Effect of host and temperature on the population structure of Puccinia striiformis f. sp. tritici, responsible of yellow rust in the Middle East

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ÉCOLE DOCTORALE 145 :
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THÈSE DE DOCTORAT

SCIENCES AGRONOMIQUES

par

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Effet de l'hôte et de la température sur la structure de la population de *Puccinia striiformis* f. sp. *tritici*, agent de la rouille jaune du blé au Moyen Orient

Date de soutenance : 25/09/2015

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Acknowledgements

I would like to express gratefully and sincerely my deepest appreciation to Dr. Claude de Vallavieille-Pope, for her guidance, understanding, and patience during my graduate study within her group. Her mentorship was paramount in exchanging her laboratory experience and tools, her research skills, personal hospitality and advices, and encouragements. Dr. Pope helped me to come up through the rough road to finish this thesis.

I do recognize Dr. Michel Afram for his constant encouragement, his wide vision in upraising the scientific level of his staff. I highly appreciate his support to have EGIDE (Agence Universitaire de la Francophonie) scholarship annually and flexibility for being many times in France to finalize the work.

Dr. Jacqui Shykoff has been supportive and showed quick assistantship over the three years academically. Dr. Shykoff came up with valuable constructive criticism to the research project; she was an amazing person and a role model with her hospitality and enthusiasm. I address my deepest appreciation and thanks to her.

I would like also to express my thanks to Dr. Kumarse Nazari for his support and for providing his facilities to launch the practical work at his laboratory at the International Center for Agricultural research in Dry Areas, Tal Hadya, Syria. I would like to thank him for offering me the opportunities to participate to the Borlaug Global Rust Initiative and to win the 2013 Women in Triticum (WIT) award.

I would like also to thank Dr. Mogens Hovmøller for his constructive, meticulous and illuminating scientific ideas on some issues related to the research project and for providing the yellow rust samples from his collection.

I would like also to thank Dr. Sajid Ali for helping in molecular works, genetics analyses and for his contribution with some illuminating ideas relevant for the research project.

Distinguished thanks and appreciation are addressed to Mr. Marc Leconte for his help with the practical work in pathotyping, aggressiveness components, and his continuous support; and to Mr. Laurent Gérard for his help and support in all practical work from planting, inoculation, and

many routine works. Many thanks are addressed to Angélique Gautier for my acceptance in her molecular laboratory and I will not forget Mme Nathalie Galet for her help in some practical work.

I would like to acknowledge Dr. Anne Marie Justensen and Dr. Stephanie Walter for her helps in genotyping the samples with SCAR markers and Dr. Bochra Bahri for her help in genetic analyses.

I would like to address my thanks for the group “Epidemiology”, BIOGER. I start with Dr. Marc Henri LeBrun, Frédéric, Ivan, Henriette, Sandrine. Many thanks to many persons those were supportive and very friendly from other groups.

I would like to acknowledge Dr. Amor Yahayoui for his encouragements and his continuous help to settle the research project and put me on the right way to start and finalize the work. Many thanks are addressed to Mrs. Maha El Ahmad for her help in spore multiplication and pathotyping of the Syrian isolates at ICARDA, Tal Hadya, Syria.

My special and very distinguished appreciations are addressed to my colleagues at the Lebanese Agricultural Research Institute; they are too many and the list is very long…

I am also grateful to Jury President and members for accepting to review and edit my work and travel along to assist to the defense.

Special and warm thanks are addressed to my family and especially to my husband and two lovely daughters for their understanding, comprehension and appreciation.

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Abstract

The adaptation of fungal pathogen to its hosts and to the climate variation, in particular to the temperature, was investigated on wheat stripe (yellow) rust, caused by the biotroph fungus *Puccinia striiformis* f. sp. *tritici (Pst)* in the Middle East, focusing on Lebanon and Syria. This disease is a major problem for the crop in the region. Specific resistance genes were postulated in 138 wheat genotypes including elite lines, grown varieties and local landraces, using an array of 11 French pathotypes. Resistance gene diversity for yellow rust in wheat elite lines was higher than in current, commercial varieties grown in Lebanon, with nine *Yr* genes detected singly or in combination. Some varieties were resistant to all tested pathotypes and might provide interesting sources of resistance. Most of the Lebanese landraces were susceptible but also heterogeneous by their number of plants susceptible and resistant to a specific pathotype in a same landrace.

A field survey was conducted in Lebanon and Syria in 2010-2011 and 275 *Pst* isolates were collected. The pathogen population was genotyped with 20 microsatellite markers and was found to be clonal, although the alternate host *Berberis libanotica* is present in the region. The dominant multilocus genotype shared similarity with the new invasive strain PstS1/PstS2 dispersed worldwide since 2000. The population was clonal with 10 pathotypes detected in Lebanon and Syria. 50 MLGs were detected considered high for clonal population. The virulence profiles combining *Vr2*, *Vr6*, *Vr7*, *Vr9,* and *Vr27* are typical of the Mediterranean area according to group (Bahri *et al.,* 2009) and corresponded to the worldwide invasive pathotype described since 2000 (Milus *et al.*, 2009). The *Vr8* was not fixed in this population, whereas this virulence is frequent in the Mediterranean genetic group (Bahri *et al.*, 2009).

Recently *Pst* strains have been described for adaptation to warm temperature (Milus *et al.*, 2009; Mboup *et al.,* 2012). The question of temperature adaptation in this study was whether the strains adapted to warm temperature are found in few clones of invasive strains or if they are selected in different pathogen genotypes locally under specific climate conditions. We selected 26 *Pst* isolates from the Middle East, 13 isolates from warm and 13 isolates from cold areas. We

assessed their infection efficiency and latent period under four temperature regimes (high and warm temperature for the spore penetration phase, and high and warm temperature for the latency period). The isolates differed for the thermal aptitude for infection efficiency and latent period, but no clear relationship was established between the climate of the origin location of the isolate and its thermal aptitude. Some isolates were able to infect at high temperature but had long latency at high temperature and vice versa, some isolates had low infection efficiency and short latent period at high temperature, and few isolates were efficient either at high temperature or cold temperature for infection efficiency. Latency period showed pattern of local adaptation. Warm dew temperatures retarded sporulation, but this effect was far less marked for isolates from warm climates when incubated under warm conditions.

This study provides details about probable effective yellow rust genes present in different genotypes and the prevalent pathotypes in the region. Moreover, the thermal aptitude for infection efficiency and latent period of some isolates under contrasting temperature will help us to build a better integrated disease management in the highlight of global warming.

GENERAL INTRODUCTION

General introduction

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1-Wheat

1. Importance in CWANA region

Wheat is a major staple cereal crop contributing directly or indirectly in substantial proportion of the caloric intake for the human population (http://faostat.fao.org). Wheat demands are increasing and by 2030 the demand for wheat (*Triticum aestivum* L.) is estimated to increase by 40% with an increase of the population of 8.27 billion (House of Commons Environment, Food and Rural Affairs Committee 2009; http://www.parliament.uk) and 60% increase by 2050. The greater demands for wheat are influenced by the population growth and dietary changes for populations. Wheat (bread and durum wheat) is consumed by 2.5 billion people in 89 countries. The total wheat production was estimated at 653 million tons in 2013 with the highest rank 695 million tons for the year 2012. Wheat is grown on 215 million hectares and distributed from Scandinavia to South America and across Asia. About 54 million hectares are grown in the Central, West Asia and North Africa (CWANA) region, including spring and winter/facultative bread wheat, durum wheat. About 50% of total durum wheat areas are located in the developing countries and 80% of the total of 11 million hectares of durum wheat is cultivated in the CWANA region. The productivity of wheat in CWANA region is lower than all geographic regions and lower than the world average of 2.9 tons/ha. In ancient times, CWANA region used to be the breadbasket of many empires. The Fertile Crescent and Turkey are considered as ones of the richest and important centers of diversity for wheat and barley. The first domesticated wheat species were einkorn (*Triticum monococcum* sp. *monococcum*) and emmer (*Triticum turgidum* sp. *dicoccon*) and then they evolved from their wild relatives (*T. boeticum* and *T. dicoccoides*, respectively) about 10 000 years ago (Heun *et al.*, 1997). Later on after its spread from the Fertile Crescent, durum wheat *(T. turgidum durum*) played a major role by providing sub-species cultivated for thousands of years across the continents (Feuillet *et al.*, 2007).

Bread wheat (*T. aestivum L.*) first emerged from an hybridization between *T. turgidum* and *T. tauschii* in cultivated wheat fields approximately 5-6000 years ago (Zohary and Hopf, 2000). The D genome encodes proteins and restores the softness of the grain endosperm of bread wheat originated from *Aegilops tauschii* (Chantret *et al.*, 2005). It carried alleles adapted to the more

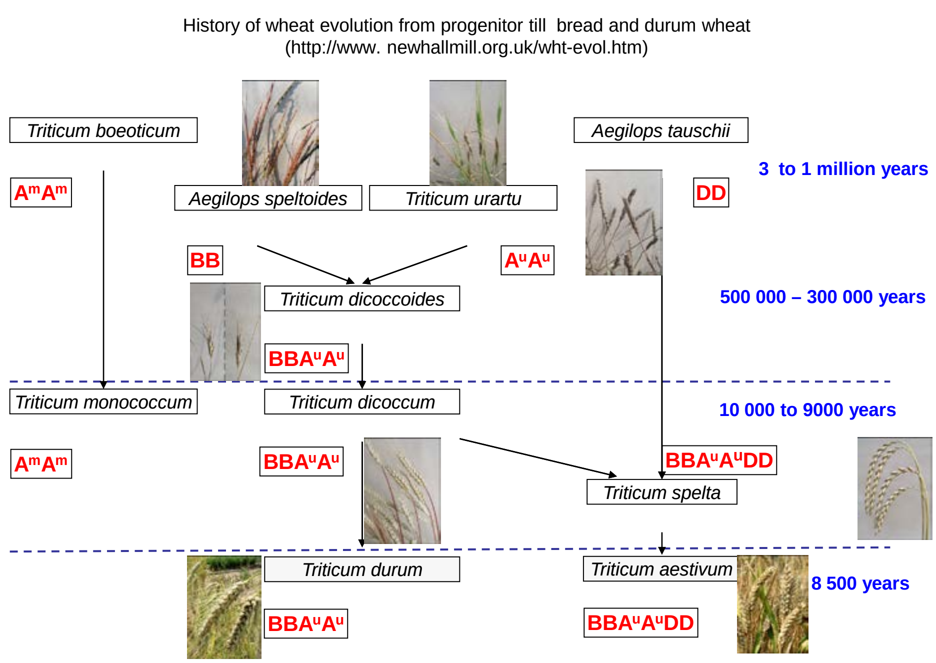

Figure 1: Simplified scheme for bread and durum wheat origin and history of evolution from progenitor (Trottet, 2011)

continental climate enabling bread wheat to be cultivated for approximately 90% of world wheat production to-day (Feuillet *et al*., 2007).

In average, the annual wheat production level in CWANA is about 126 million tons on the total area, and is far below the regional demand of about 164 million tons. However, the highest wheat consumption is registered for this region and is about 185 kg/capita/year. Moreover, CWANA and sub-Saharan Africa will be the most adversely affected with the climate change resulting in decrease in cereal production and increase in irrigated cereal production (ICARDA, 2010). Beside the abiotic stresses affecting the region, wheat is subjected to many biotic stresses.

1. 2 Taxonomy and phylogeny

Wheat (*Triticum aestivum*) belongs to *Magnoliophyta* divison, *Liliopsida* class, order Cyperales, family *Pooideae* which is the fourth largest cultivated among flowering plants. In this family there are some of the most important crops such as maize (*Zea mays*) and rice (*Oryza sativa*). Wheat is superior to most other cereals in their nutritive value. Grass genomes differ in size, ploidy, and chromosome number. Archeological findings had highlighted that wheat was domesticated 10.000 years ago in the Fertile Crescent that extends from the Eastern part of the Mediterranean to the lower Zargros Mountains in Iran and Iraq (www.icarda.org). Bread wheat originated through polyploidization events (Feldman *et al*., 1995; Huang *et al*., 2002). The three ploidy are: the diploid Einkorn (2n = 14), the tetraploid such as Emmer and *T. turgidum* L. (2n = 28) and the hexaploid, spelt and *Triticum aestivum* L. (2n = 42).

The current cultivated bread wheat is an allohexaploid (2n = 6x = 42), has the AABBDD genome and is characterized with the largest genome 17 Gb. The genome is identified with three groups of seven chromosomes. A set of three pairs of homologous chromosomes A, B, and D is present in each group. The genome AA, BB, DD derived from *Triticum urartu* and *Triticum boeticum*, *Triticum turgidum dicoccoides*, and *Aegilops tauschii*, respectively (Fig. 1). The A genome comprises the highest number of transposable elements compared to B and D genomes (Sabot *et al.*, 2005).

1. 3 Landraces versus improved varieties

In 1975, Harlan defined the landraces as the principal focus of agricultural production (Newton *et al*., 2010). The Fertile Crescent is known as one of the important centre of diversity of many field crops, particularly wheat and barley. Recently, Camacho Villa *et al*. (2005) came out with a new definition "a landrace is a dynamic population(s) of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems". In modern farming, the landraces have been replaced by elite cultivars. Elite cultivars are derived from a relatively narrow germplasm pool and are well adapted to high input agriculture. Landraces are known for their diversity and heterogeneity. Zeven (1998) described nicely the "yield stability of landraces under traditional low input agricultural system is due to the fact that whatever the varying biotic and abiotic stress for each plant, one or more genotypes within the landrace population will yield satisfactorily". Landraces have closer affinity with modern cultivars than wild species and can be eventually as a foundation material in breeding programs. Recently, many studies were conducted on physiological, biochemical, molecular and technological traits on bread wheat landraces (Newton *et al*., 2010). They reviewed the status of landraces and their context in sustainable agriculture and highlighted major points as: 1) Landraces have diverse response to many stresses and are large resources for the development of future, 2) Worldwide, many germplasm collections of landraces are available and have not been characterized, 3) The germplasm materials have been maintained ex-situ and in situ where they evolve and both methods have their advantages and disadvantages, 4) The high quality phenotyping-genotyping association enables to exploit the variations present in the landraces, 5) Landraces display a potential source of traits for crop improvement particularly those related to developmental stage, soil condition and root system, 6) Landraces are rich with nutritional traits such as antioxidants, phenolics, carotenoids and tocols; Zinc and iron have been successfully transferred to improved varieties, 7) Landraces have potential sources of disease tolerance and resistance of pest and various abiotic stresses.

2- Biology of *Puccinia striiformis* f. sp. *tritici* (*Pst*)

2.1 Classification

Rust fungi are cosmopolitan plant pathogens, well distributed on a wide host range, including ferns and conifers, and most families of dicotyledon and monocotyledon angiosperms. The rusts belong to the family *Pucciniaceae*, Order *Pucciniales*, Class *Uredinales*, and *Basidiomycota* phylum. Rust fungi are obligate biotrophic parasites, depending entirely on living host cells to complete their biological cycle (Cummins and Hiratsuka, 2003). *Puccinia* is one of the main genera accounting for 3000-4000 species that are highly diverse with respect to host preference and number of spore stages reaching up to five within the life cycle (Staples, 2001; Liu and Hambelton, 2010). *Puccinia striiformis* infects at least 320 grass species in over 50 genera from the *Poaccae* family (Hassebrauk, 1965). Eriksson was the first to use forma specialis of *P. striiformis*; Liu and Hambleton (2010) summarized the host range of *P. striiformis* and cited the following: *P. striiformis* f. sp. *tritici* infects *Triticum aestivum*, *P. striiformis* f. sp. *hordei* infects *Hordeum vulgare*, *P. striiformis* f. sp. *pseudo-hordei* infects wild barley grass (*Hordeum* spp.), *P. striiformoides* infects cocksfoot grass (*Dactylis glomerata)* and *P. pseudostriiformis* infects mainly Kentucky bluegrass (*Poa pratensis*).

The complete genome of *P. striiformis* is estimated to be 110 Mb (Zheng *et al*., 2013). Comparing to the conventional sequencing technologies, the Next Generation Sequencing (NGS) has been improved speedily in term of efficiency and cost reduction (Cantu *et al*., 2011). For instance, Saunders *et al*. (2012) used high throughput computational methods to characterize the effector complements from the fully sequenced rust fungi *Puccinia graminis* f sp. *tritici* and *Melampsora larici-populina*. Cantu *et al*. (2011) provide a draft genome of a *Pst* isolate (PST-130), annotating 22,185 putative coding sequences and classified 1,088 of these as predicted secreted proteins. Moreover, a BAC library (Chen & Ling, 2004), a cDNA library from uredospores (Ma *et al.*, 2009), a germinated uredospores EST library (Zhang *et al.*, 2008), and a haustorial EST library (Yin *et al*., 2008) have been identified and publicly available.

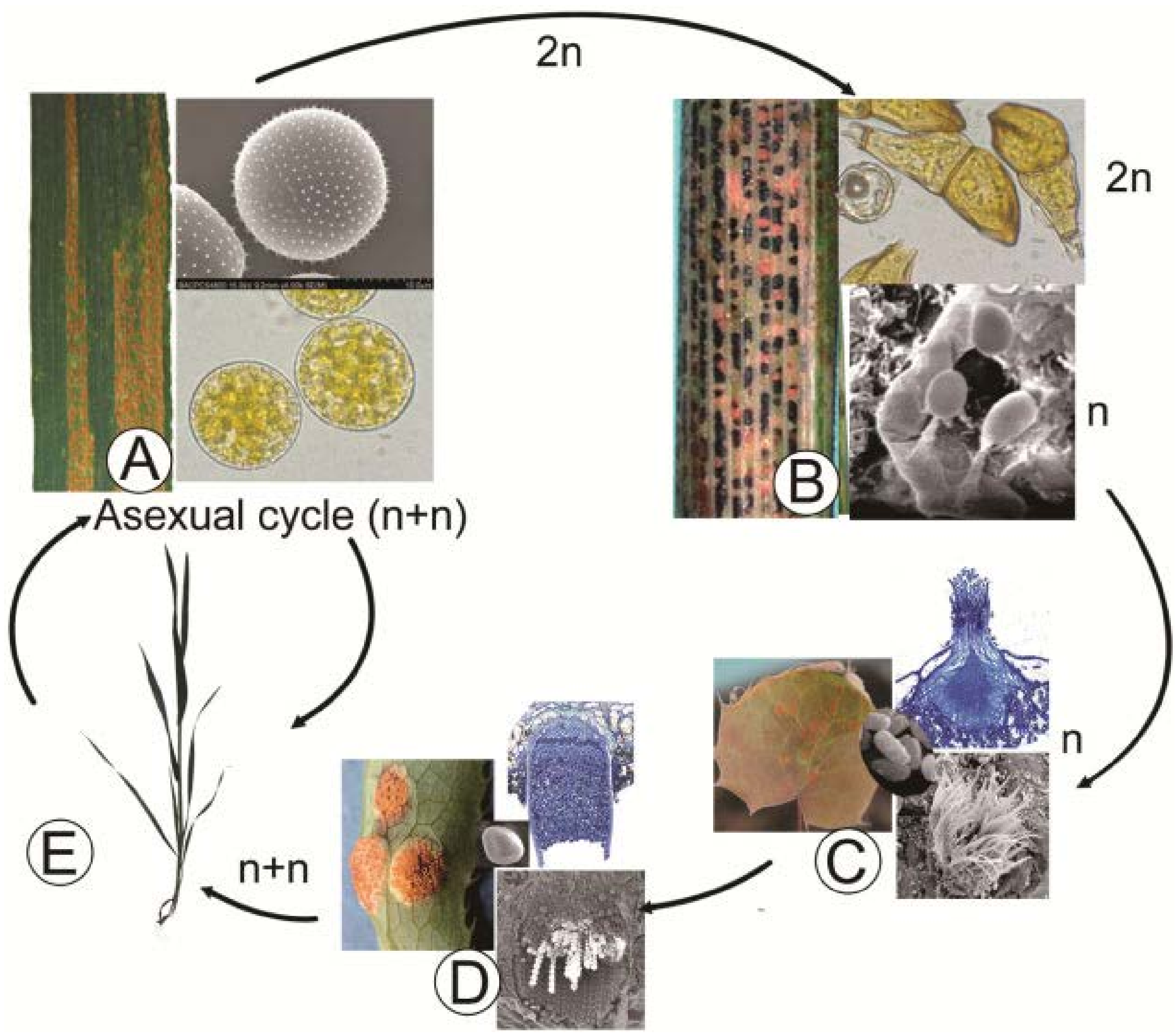

Figure 2: Life cycle of *Puccinia striiformis* f. sp. *tritici*. (A) Uredinia on wheat leaf containing single-celled dikaryotic uredospores (n + n) originating from aeciospores (n+ n) or urediniospores. Top inset: echinulate surface of a uredospore under a scanning electron microscope (SEM) (×4000). Bottom inset: broadly obovoid uredospores (×1000). (B) Telia typically form beneath the leaf epidermis near the end of the growing season. Top inset: the two-celled, oblong-clavate teliospores (2n) (×1000). Bottom inset: the elliptoid basidiospores (n) from the germination of teliospores (×2500). (C) Pycnia produced by basidiospore infection on *Berberis chinensis* on upper leaf surfaces via inoculation with germinating teliospores of *P. striiformis*. Top inset: a magnified flask-shaped pycnia (×400). Middle inset: the oblong-shaped pycniospores (×4000). Bottom inset: magnified receptive hyphae (×900). (D) Cluster of sunflower-shaped aecia produced on the lower leaf surface of *Berberis shensiana*. Top inset: a campanulate aecium (×200). Middle inset: flat spherical-shaped aeciospores (×3300). Bottom inset: cluster of aeciospores (×250). (E) A wheat seedling that can be infected by aeciospores produced on barberry plants and can produce uredospores (Chen *et al*., 2014).

2.3 Life cycle

The causal pathogen *Puccinia striiformis* Westend. f. sp. *tritici* Erick. (*Pst*), is one of the most widely destructive plant disease to present international wheat production (Wellings, 2011) as well to old times to the Bible, to the Greek and Roman Empires (McIntosh *et al.,* 1995). *Pst* is classified as a biotrophic obligate parasite relying on the host plant to complete the life cycle. *Pst* was classified as an autoecious microcyclic rust pathogen, has been considered until recently to reproduce only asexually through dicaryotic uredospores on wheat, the basidiospores produced by teliospores produced at the end of the cropping season failing to encounter an alternate host to finalize the sexual cycle (Fig. 2). Recently Jin *et al*. (2010) and Rodriguez-Algaba *et al.,* 2014 showed that *Pst* completes its sexual cycle on different species of *Berberis,* in controlled conditions but the role of the sexual cycle has never been shown under natural field conditions. In the conditions of Pacific Northwest of the United States, Wang & Chen (2015) showed that there is no synchrony in the prevalence of susceptible leaves of *Berberis* and phenology of *P. striiformis*, i.e. the period when basidiospres are released. Furthermore, Kang *et al.* (2015) found very few *Pst* aecia on *Berberis* in China. Under natural conditions, sexual reproduction was suspected to occur in populations that exhibit footprints of recombination in their genetic structure, such as in China, Nepal and Pakistan (Ali *et al*., 2014b). Algaba *et al*. (2014) established a successful life cycle of *P. striiformis*, using *Berberis vulgaris* as alternate host under laboratory conditions.

2.4 Symptoms and economic losses

Pst is the most serious biotic threat to sustainable international wheat production (Wellings, 2007), and has been the important disease constraint to winter wheat in Central and West Asia over the last 12 years (Nazari *et al*., 2008; Ziyaev *et al.*, 2011). The fungus *Pst* infects green tissues of cereals and grasses crops. Symptoms appear like tiny, yellow- to orange-colored rust pustules, called uredia. Each uredium contains thousands of uredospores. Spores appear as a powdery mass differing in color from yellow to orange color (Chen, 2005). At the senescence and late growth stage, black telia are often produced (Fig. 3). Yield losses in wheat from *Pst* infections are arising from reduced kernel number per spike, low test weight and reduced and shriveled kernel quality (Prescott *et al*., 1986; Roelfs, 1978).

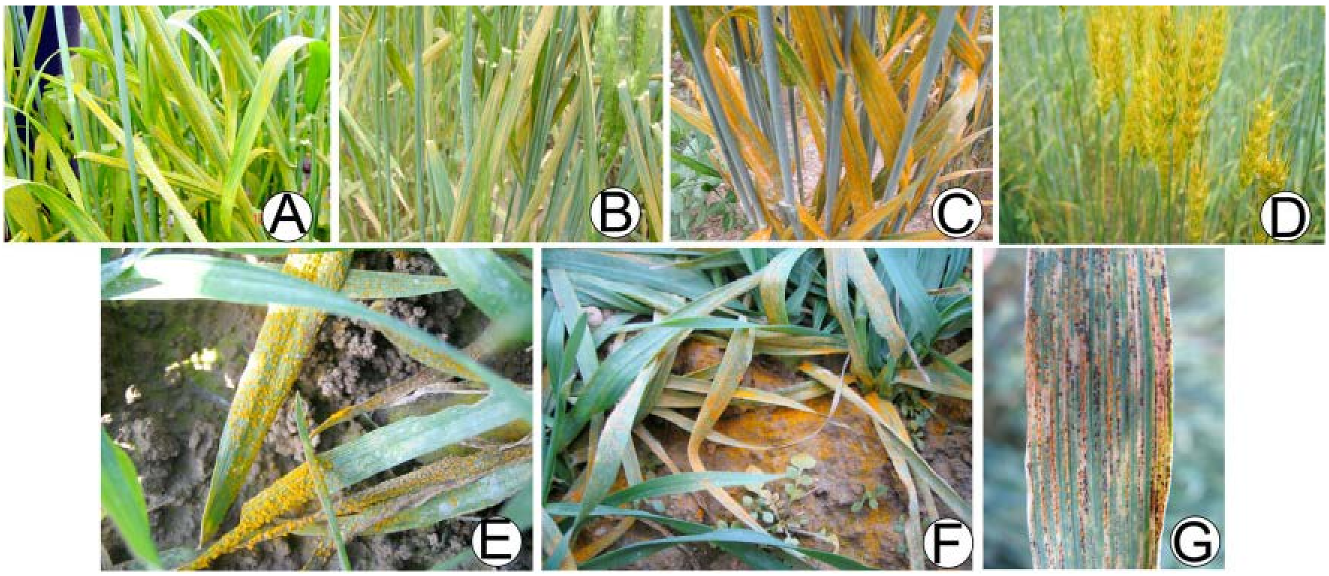

Figure 3: Wheat stripe rust symptoms in the field. (A–C). Yellow to orange uredinial pustules on susceptible adult plant leaves. (D) Uredinial pustules on the glumes and awns. (E, F). Yellow to orange uredinial pustules on the seedling wheat leaves. (G) Telial and uredinial pustules together on an adult plant leaf (Chen *et al*., 2014).

In Australia, the yield losses due to yellow rust, if the disease was not controlled, was estimated up to A$83-43 per hectare and A$994 million at the national level (Murray & Brennan, 2009).
In the United States, annual losses on wheat exceeded one million metric tons over several years since 2000 and mounted to 2.4 million tons in 2003 (Long, 2009). In China, yellow rust annual losses varied between 1.8 and 6.0 million tons in three epidemic years (Wan *et al*., 2004). In the year 2009, the yield losses reached the highest records in Northern Africa (Ezzahiri *et al*., 2009) where 90% of the varieties showed susceptibility to the disease.

3. Quantitative components of host-pathogen interaction

Quantitative components of the pathogen life cycle include germination and infection efficiency, latent period, sporulation rate, infectious period and lesion size.

3.1 Germination and infection efficiency

The climatic conditions play a major role in the potential of a spore to infect and germinate. Uredospores germinate between just above 0°C and 20°C, but the optimum temperatures for germination are from 7°C to 12°C (Chen, 2013). Spores produced between 5°C and 10°C

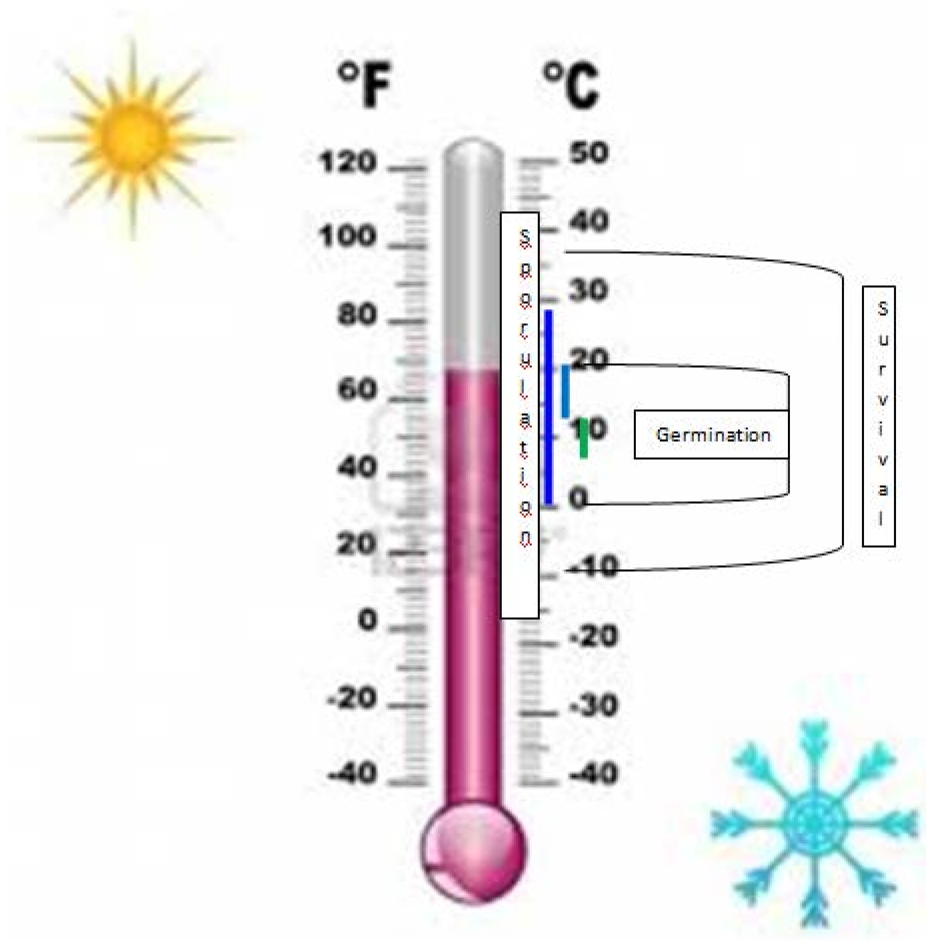

Figure 4: The lethal survival temperature are below -10°C and above 37°C. Germination temperature is 0-20°C and optimal from 7 to 12°C (Green highlight) The optimal temperature range for sporulation is 12°C to 20°C (Light blue line) but can occur from just above 0°C to 28°C (Dark blue highlight) (Modified from Chen *et al*., 2013).

germinated best in the presence of dew whereas spores produced at 30°C and over were unable to germinate (Georgievskaja in Rapilly, 1979). Schmitt *et al.* (1964) considered a temperature range from -2.8 to 21.7°C with the optimum 9.7°C whereas de Vallavieille-Pope *et al.* (1995) demonstrated for a Northern French isolate that the range of infection is at 5-12 °C during the dew period. Under free water conditions at 12°C, uredospore germination initiates after 3 hours with contact of free moisture; uredospores germinate diving 24 hours to produce a promycelium of four cells (Fig. 4) (Chen, 2013). Infection efficiency is the percentage of successful infections after the deposition of controlled number of spores (Metha & Zadoks, 1970). Practically, due to the coalescence of the sporulating lesions growing on the leaf, this parameter is evaluated by counting the chlorotic flecks per unit of leaf area (Milus & Line, 1980) and expressed as the percentage of spores which produce sporulating lesions. *Pst* uredospores have a semi-systemic growth within the leaf, developing sporulating stripes. Therefore, the point of infection of several spores might overlap, and to distinguish the success of each spore, the infection success is scored at early stage with the chloroses. Infection efficiency was considered quite low for *P. striiformis* (ca 1-4%) (Hau & de Vallavieille-Pope, 2006) but if the plants received light before infection, infection efficiency can reach 30-40% (de Vallavieille-Pope *et al*., 2000) and be as high as for other wheat rusts. Isolates can differ for their thermal aptitude in term of infection efficiency. Loladze *et al.* (2014) compared the stripe rust pathogen population dominant in Australia since 2002 to previous pathogen populations for latent period and infection efficiency. The new Australian pathotype 150 E16 A+ had the highest percentage of infected plants (71.6%), but was not significantly different ($P < 0.05$) from five of the old pathotypes. Variation in latent periods has been found among American and Australian isolates (Loladze *et al*., 2014; Milus *et al*., 2006). In the US states at East of the Rocky Mountains, new *Pst* races completely replaced the old races that were found before 2000. All new isolates differed from the old isolates by showing shorter latent periods at 18°C than at 12°C (Milus *et al*., 2006). Latent period is expressed in degree-days for Septoria leaf blotch on winter wheat, but Milus *et al.* (2009) evaluated the latent period for *Pst* isolates in days.

Sporulation corresponds to the contagious period (Rapilly, 1979) and is evaluated with the rate of sporulation. This rate is the amount of spores produced per lesion and per unit of time (Sache, 1997). The sporulation occurs when relative humidity is not exceeding 50%. However, free water droplets stop sporulation (Rapilly, 1979). According to Newton & Johnson, (1936) the

			Host genotype	
			Resistance	Susceptibility
			RR	rr
Pathogen	Avirulent	AA	AR (-)	Ar (+)
Genotype	Virulent	aa	aR (+)	Ar (+)

- = Resistance or incompatible reaction

+ = Susceptiblity or compatible reaction

Figure 5: Quadratic check for Gene-for-gene hypothesis.

optimal temperature range for the latent period is 13–16 °C. The optimal temperature range for sporulation is 12°C to 20°C but can occur from just above 0°C to 28°C (Chen, 2013).

3.3 Lesion and density size

The lesion size is defined as the surface area that produces spores. This parameter is not easy to assess especially for *Mycosphaerella graminicola (Zymoseptoria tritici)* that induces necrosis on the host leaf (Cowger & Mundt, 2002), but in case of *Puccinia triticina*, causal agent of brown/leaf rust, lesions are limited, but it can dramatically increase in *Pst* for which lesion growth is semi-systemic and subsequently difficult to assess (Schmitt *et al.*, 1964).

4- Host-pathogen interaction

4.1 Principles of gene-for-gene (GFG) concept

Understanding how pathogens harm the plants is a primordial focus in plant pathology and is of particular importance for cultivated host plants. Among plant diseases, wheat yellow rust was the first to be studied for genetics of resistance. In 1905, Biffen described the first Mendelian resistance to *Pst* in the wheat variety Rivet (Knott, 1989). This finding was the causal factor for understanding the host-pathogen interaction (De Wit, 1992). In 1947, Flor studied the inheritance of host reaction and pathogenicity of *Melampsora lini* in *Linum usitatissimum* L. and proposed the gene for gene (GFG) hypothesis and expressed in incompatibility between a host and a pathogen involved corresponding genes in each organism. This incompatibility is the result from the interaction between a race specific receptor in the resistant host and a race specific elicitor in the avirulent pathogen. For expression of incompatibility or resistance, at least one R gene in the host should recognize the corresponding avirulence (Avr) effector gene in the rust pathogen. Each R gene confers resistance to pathogen strains carrying the corresponding avirulence effector (Avr) gene. Four well-known quadratic checks for GFG interactions describing effector-triggered immunity of host and pathogen (Fig. 5). To better set the host-pathogen interaction, Loegering & Powers (1962) proposed a terminology: resistant or susceptible reaction of the host; virulent or avirulent pathogen; and the interaction results in an infection type which may be low or high. McIntosh & Wellings (1986) revised the gene for gene concept for wheat rust and proposed the following rules: the first rule is addressed to the interaction of products of single genes in hosts and pathogens, whereas the second is addressed to the second order of interaction, epistasis of resistance reaction over susceptible one. Formally stated:

1. Incompatibility between a host and pathogen is the consequence of interaction between the products of at least one host resistance gene and at least one corresponding pathogen avirulence gene, that is: *LIT = LP:LR.*
   where: LIT is low infection type*;* LP is low pathogenicity*;* and LR is low reaction*.*

2. When more than one interacting gene pair are involved, the level of incompatibility is as low as, or lower than the level produced by the most incompatible interacting gene pair acting alone, that is: LIT1,2 $\leq$ LIT1 where: LIT1 $<$ LIT2.

In host pathogen interaction, McIntosh & Wellings (1986) discussed four familiar applications based on the gene-for-gene hypothesis:

1. Unknown host and unknown pathogen.
2. Known host and-unknown pathogen applied in case of race analysis studies and pathogenic variation.
3. Unknown host and known pathogen applied in case of gene postulation.
4. Known host- known pathogen applied in fundamental studies of host pathogen interactions.

4.2 Molecular approach

Many R genes are race-specific and confer resistance during the seedling stage. Identification and functional analyses of these genes are crucial in understanding the resistance mechanism. R genes are encoding receptors to detect the presence of the pathogen and activating signaling cascade leading to resistance reaction (Hammond-Kosack & Jones, 1997). R genes are classified in five major groups. The first group characterized by the presence of a nucleotide binding site

(NBS) and leucine rich repeat (LRR). The NBS region is related with plant cell death (Biezen & Jones, 1998). Three separate functions characterize the NB-LRR protein: (1) pathogen strain (effector) recognition, (2) R protein transition from the resting to active state and (3) signaling to the host defense response machinery. The Pto group is characterized by a Serine/threonine kinase with N-terminal site. The Toll-Interleukin Receptor/Coiled coil (TIR/CC) group includes a nucleotide binding site (NBS), a leucine rich repeat motif plus either a coiled coil (CC) sequence or a Toll and Interleukin-1 receptor type region (TIR). These two groups are localized intracellular. The LRRs and LRR kinase group are trans-membrane protein with extracellular LRRs. The LRR kinase has a cytoplasmic protein kinase domain. The fifth group, the Single Anchor/Coiled coil (SA:CC) group which carries a putative signal anchor (SA) for membrane insertion, and a putative CC domain (Dangl & Jones, 2001; Jones, 2001). Saunders *et al.* (2012) identified diverse set of candidate effectors, including families of haustorial expressed secreted proteins and small cystein-rich proteins for *Puccinia graminis* f. sp. *tritici*, and *Melampsora larici-populina*.

5- Co-evolution and population dynamics of *Pst*

5.1 Center of origin and diversity of *Pst* populations at the global level.

For a long time, the center of diversity of *Pst* has been thought to differ from the center of domestication of cereals, that is the fertile crescent. Transcaucasia has been hypothesized by Hassebrauk (1965) and later by Stubbs (1985) to be the center of origin of *Pst* based, on disease and pathotype prevalence and geographical barriers. The recent findings on the population structure of *Pst* showed very high diversity in the Himalayan zone (Ali *et al.*, 2014b) with production of lot of telia of isolates from this area in laboratory conditions (Ali *et al*., 2010) suggesting that Himalayan zone is the new center of diversity for *Pst*. Despite this long-distance migration capability (Brown & Høvmoller, 2002), *Pst* population structures diverged highly at a worldwide scale as shown by independent national surveys using different genetic markers. The divergent Northern population was compared to North-African and Middle-Eastern populations in 2005 and 2006. Fourteen multilocus genotypes

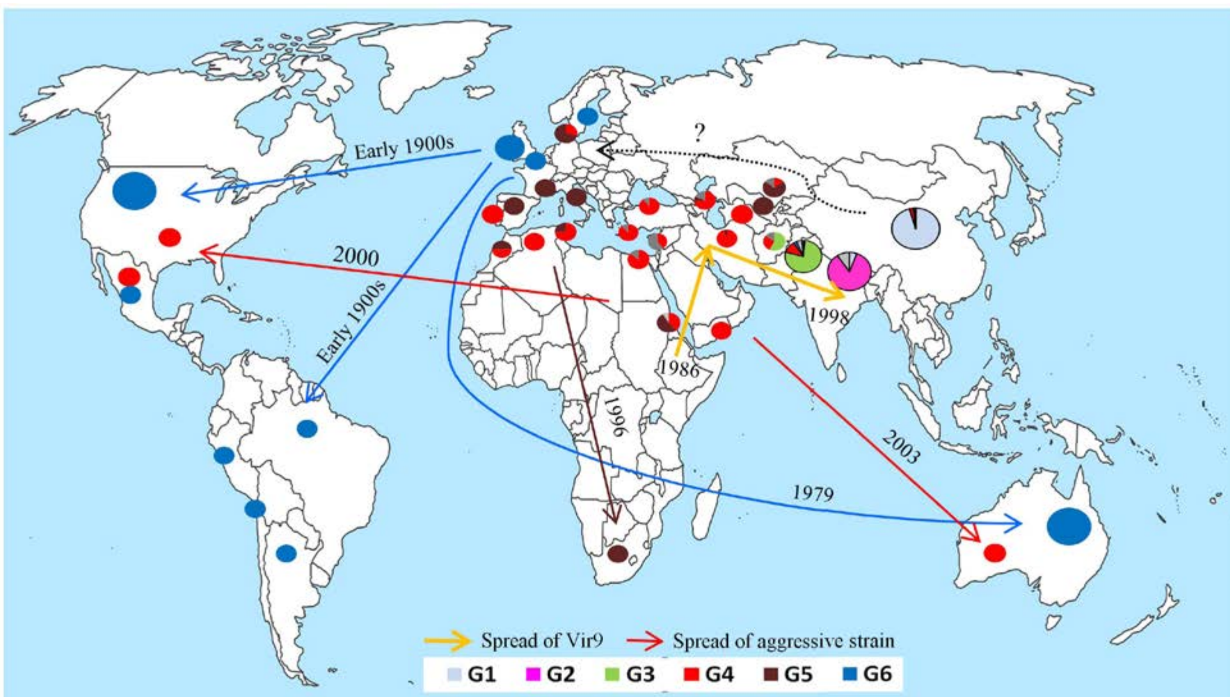

Figure 6: Origin and migration routes of recently emerged populations of wheat yellow rust pathogen identified or confirmed through population genetic analyses of a worldwide representative set of isolates. G1 to G6 represent the six major genetic groups (Ali *et al*., 201b).

obtained from 16 microsatellite markers were clustered in 3 genetic groups. The North-West European group strongly diverged from the other 2 groups with 71% of the observed SSR polymorphism. The Mediterranean group included the pathotype 6E16 found up to 2002 in Southern France and rare in the West-Mediterranean area. 6E16 differed from the two dominant pathotypes in North Africa for four virulences and several molecular markers, they were determined as the aggressive PstS2 strain. This group differed only for 14% of the observed SSR polymorphism from the Middle-eastern group. In the Middle-East, the diversity was higher with nine pathotypes, one very close PstS2 strain which was likely to have originated in the Middle East.

Populations were clonal in USA, Australia, New Zealand, and Europe and diverse in Pakistan and China (Ali *et al*., 2014). The world population structure showed evidence of geographic structuring, with six major genetic groups (Fig. 6) from the set of multilocus genotypes obtained with 20 SSR markers. These 6 groups were associated with their likely geographical origin. The Chinese population was clearly separated from Middle-Eastern, Mediterranean and Central Asian populations. This cluster was further divided into two groups, one specific to the Middle-East and East-Africa, and another group specific to the Central-Asia and Mediterranean regions. A Pakistan-specific group was distinguished. NW European populations were separated from the Chinese population, and a Nepali group was individualized. More recently, two high temperature-adapted aggressive strains closely linked genetically and named PstS1 and PstS2, are spread worldwide. Strain1, first detected in 2000 in USA, has spread quite rapidly in that country and Australia at high frequencies. Strain2 has spread in Asia, North Africa, Middle East, and Europe. East Africa has both strains (Hovmøller *et al.*, 2011). PstS1 and PstS2, were assigned to the Middle Eastern-Red Sea area-East Africa group, suggesting this area as the source for these widely spread, aggressive strains. An older set of aggressive isolates frequently reported in Europe and Mediterranean area named 6E16, although probably less aggressive than PstS1 and PstS2, was assigned to the Central Asian-Mediterranean genetic group (Ali *et al.,* 2014b).

The rapid evolution of virulence in the Northern French population is driven by the acquisition of new virulences in resident clonal lineages, in response to major resistance genes deployed locally. A stepwise clonal evolution based on AFLP markers and virulences can explain the Northern French population evolution over the last 25 years (de Vallavieille-Pope et al., 2012). Hubbard et al. (2015) observed a shift in the *Pst* population structure observed in the UK recently and identified four distinct lineages that correlated to the phenotypic groups by applying a field pathogenomics approach by transcriptome sequencing infected wheat leaves. In 2011, new multivirulent strains arose in UK, France, Denmark and Sweden. Based on PCR, these strains may be migrants (www.wheatrust.org).

In China, Shan *et al.* (1998) showed high genetic diversity, especially in Tianshiu county of the Gansu province, using repeated genomic sequences. Duan *et al*. (2010) found 139 AFLP genotypes among the 160 isolates analysed. The factorial correspondence analysis on AFLP data did not reveal different genetic structures among the 5 populations. The absence of linkage disequilibrium in the AFLP data shows that Gansu populations were not strictly clonal, contradicting previous findings, and demonstrated that recombination must occur in addition to the epidemic clonal behavior of this species. The marked diversity has been attributed to the specific environmental and agronomic conditions that favor inter-epidemic survival of the fungus. The differences in wheat maturation between the altitudes induce an overlapping period between earlier sowing and harvest periods in the lowlands than in the highlands. This green-bridge was expected to maintain higher genetic diversity by reducing recurrent inter-season demographic bottlenecks. Diversity was not due only to reduced bottlenecks in the oversummering area. The high pathotype diversity was also observed in the Gansu area with 86 pathotypes within a subset of 140 isolates, in contrast to the low diversity in other regions such as France, where only 22 pathotypes were detected among 1 334 isolates collected over a 25 year-period. There was no correlation between pathotypes and MLGs. The lack of linkage disequilibrium observed between virulence factors and molecular markers also agreed with random mating.

World population structure showed that geographically distant populations were genetically related and provided evidence of intercontinental migrations. The distribution of the NW European genetic group confirmed NW Europe as the source of the North American populations in the early 1900s. *Pst* was also first reported in South America in the early 20th century and has the same NW European origin, revealing another incursion from NW Europe. In 1979, *Pst* was introduced from NW Europe into Australian populations. The Mediterranean and Central Asia group appeared to be the source of the population in South Africa, where *Pst* was absent before 1996. Boshoff *et al.* (2002) showed that the first pathotypes detected were similar to those present in the Middle East and Mediterranean regions. The widespread race in Europe since 2011, "Warrior" race, was detected in Morocco and Algeria. This race attacks both wheat and triticale, showed the same aggressiveness as PstS1 and PstS2 but reported earlier (www.wheatrust.org). Aggressive strains PstS2 were detected in 2014 in East Africa and Asia. The virulence profile comprises *Vr2, 6, 7, 8, 9* combining often *Vr*27. The PstS2 strains was detected in Ethiopia, Kenya, Tanzania, and Rwanda with additional virulence to *Yr1* and *Yr10* (www.wheatrust.org). Centers of diversity may contribute to emergences of new strains but other significant drivers of population structuring are those due to temperature and deployment of resistance genes.

5.2 Adaptation to temperature and effect on *Pst* population

The *Pst* worldwide structure cannot be explained only by resistance gene deployment. Another driver, in recent years, temperature, was advocated to contribute to population structuring (Milus *et al*., 2009; Mboup *et al*., 2012). Based on molecular markers and pathotypes, Enjalbert *et al.* (2005) have shown a strong and steady spatial structure between Northern and Southern French populations. Northern pathotypes, which carried all the virulences necessary to infect Southern cultivars, were not selected in the South. There was a climate effect, the temperature being higher in the south than in the north. The Southern isolates, which belonged to a Mediterranean genetic group, were more adapted to high temperatures (Mboup *et al*., 2012). The French isolates germinate equally at low temperature, but the Mediterranean

Southern isolates have higher germination rates at high temperature. At low temperature, Northern European isolates have higher infection efficiency, but at high temperature, 20°C, only Southern French isolates were able to infect the wheat cultivar. At high temperature, the latency was shorter for Southern isolates by 0.8 day compared to the Northern isolates.

The Southern Mediterranean isolates produced two times more spores per leaf than the north-western European isolates at high temperature. At all studied stages of the infection process, the Southern Mediterranean genotypes were found to be more adapted to high temperature than were the West-European ones. The advantage of the Southern isolates at high temperature was confirmed by isolate competition in the field, at the adult plant stage and over several infection cycles (Mboup *et al*., 2012). An equal proportion of spores of the Southern and the Northern isolates were inoculated in field plots in the north and south of France. Frequency of the Southern isolate at the end of the season was assessed by its infection efficiency on a differential cultivar. The final frequencies of Southern isolates are higher than the initial 50%, both in the north and south experimental fields. For the four different Southern/Northern pairs of isolates tested, the mean competitive success of southern isolates was significantly higher in the southern than in the northern location in most cases. The field experiment revealed competitive superiority of Southern over Northern isolates, especially in the Southern assay location. Evidence for differential response of pathogen genotypes to temperature, in accordance with their region of collection was shown.

Adaptation to warm temperature was also tested by Milus *et al*. (2009) with North American isolates, by comparing the new aggressive isolates belonging to the Middle Eastern genetic group to the old isolates belonging to the NW European group**.** Milus *et al.* (2009) demonstrated on adult plants that the strains spread all over the world since 2000 were better adapted to warmer temperatures than are old isolates. The new PstS1/S2 strains sporulated 2.1 days sooner at the low temperature, and 3 days sooner at high temperature than the old isolates. They also produce 2 to 3 times more spores per day compared with old isolates. New PstS1 and S2 isolates showed significant adaptation to the warm temperature regime for all variables of infection cycle. These differences may have contributed to the recently expanded geographic range for *P. striiformis*. Nowadays, stripe rust epidemics occur in areas once believed too warm for *Pst* growth and reproduction, like in the Southern United States and West Australia.

Table 1: List of genes for resistance to *Puccinia striiformis* f. sp. *tritici*, chromosomal locations, source of the gene and types of resistance (www.shigen.nig.ac.jp/wheat/komugi/genes).

Yr gene	Chromosomal location	Germplasm source
*Yr*1	2AL	Chinese 166
*Yr*2	7B	Kalyansona, Heines VII
*Yr*3	Undesignated allelle	Vilmorin 23, Minister
*Yr*3a	1B, 2BL	Nord-Desprez, Vilmorin 23, Cappelle Desprez
*Yr*3b	Unknown	Hybrid 46
*Yr*3c	1B	Minister, Maris Beacon
*Yr*4	3BS	Avalon, Rubric, Bolac, Emu S
*Yr*4a	6B	Cappelle Desprez
*Yr*4b	6B	Hybrid 46
*Yr*5	2BL	*Triticum aestivum* Spelta Album
*Yr*6	7B	Heines Kolben
*Yr*7	2BL	*Triticum durum* cv *Lumillo*, Lee, Thatcher
*Yr*8	2A or 2D	*Aegilops comosa*, Compa ir
*Yr*9	1BS	*Secale cereale* cv. Petkus, Clement, Kavkaz,Riesebel 47/51
*Yr*10	1BS	Spelt wheat 415, Moro, Turkish line PI 178383
*Yr*11	Unknown	Joss Cambier, Heines VII
*Yr*12	Unknown	Frontier, Mega
*Yr*13	Unknown	Hustler, Maris Huntsman
*Yr*14	Unknown	Hobbit, Kador, Maris Bilbo
*Yr*15	1BS	*Triticum dicoccoides* accession G25, Boston, Cortez
*Yr*16	2D	Bersée, Cappelle Desprez
*Yr*17	2AS	*Aegilops ventricosa*, VPM1, Rendez-vous, Brigadier
*Yr*18	7DS	Frontana, Jupateco R
*Yr*19	5B	Compair
*Yr*20	6D	Fielder
*Yr*21	1B	Lemhi
*Yr*22	4D	Lee
*Yr*23	6D	Lee
*Yr*24	1BS	*Triticum turgidum*
*Yr*25	1D	TP 129, Strubes Dickkopf, Heines Peko, Heines VII

There is a question whether there is a correlation between adaptation to warm temperature and the genetic background because all tested isolates not adapted to warm temperature belong to the NW European genetic group while those adapted to warm temperature belong to Mediterranean or Middle East genetic groups. Both temperature and resistance genes are drivers leading to divergence between the clonal lineages, and there is a need to consider agro-climatic conditions when developing strategies to improve durability of resistances.

5.3 Resistance genes

Genetic ways to withstand the pathogen populations were often based on specific *Yr* genes, which follow the gene-for-gene model. Their virulences were found few years after their release in wheat cultivars due to the rapid evolution of new pathotypes. Up to date, 70 yellow rust resistance genes had been catalogued and the details are given in Table 1 (www.shigen.nig.ac.jp/wheat/komugi/genes). These resistance genes were often considered to be inefficient. However, the combination of some *Yr* genes, including *Yr1*, *Yr3, Yr4*, and *Yr17* still protects NW European cultivars against the invasive strains PstS1/S2 adapted to high temperature (de Vallavieille-Pope *et al.*, 2012). Furthermore, the specific resistance genes *Yr7* and *Yr17*, which were overcome rapidly when used alone on large scale (Bayles *et al*., 2000), were shown to be present in cultivars having durable resistance, as *cv*-Apache, the most widely cultivar in France the last decade (Paillard *et al*., 2012). Four French wheat cultivars combining genes for specific resistance and partial have remained effective against yellow rust for decades and can be considered as sources of durable resistance to-day, cv-Renan (Dedryver *et al.*, 2009) and cv-Camp Rémy, (Mallard *et al*., 2005). One to 5 QTLs in addition to seedling *Yr* genes were sufficient to provide a high level of resistance.
Another way is to organise the diversity of resistance genes within a field. A recent review of Huang *et al.* (2002) based on 133 cases of field data on use of cultivar mixtures for controlling stripe rust showed that most of them produce positive mixture effects. Disease severity progression in the field showed that one third of susceptible cultivar was well protected by two

*Yr*26	1B	*Triticum turgidum*
*Yr*27	2BS	Selkirk, Ciano 79
*Yr*28	4DS	*Aegilops tauschii*
*Yr*29	1BL	Pavon 76
*Yr*30	3BS	Opata 85, Parula, Pavon 76
*Yr*31	2BS	Pastor
*Yr*32	2AL	Carstens V, Tres
*Yr*33	7DL	Batavia
*Yr*34	5AL	WAWHT2046=AUS91389
*Yr*35	6BS	Triticum dicoccoides 98M71
*Yr*36	6BS	Triticum dicoccoides ,RSL 65, UC1041
*Yr*37	2DL	*Aegilops kotschi*, Line S14
*Yr*38	6A	*Aegilops sharonensis*, Line 0352-4
*Yr*39	7BL	Alpowa
*Yr*40	5D	*Aegilops geniculata*, TA5602, Chinese
*Yr*41	2BS	Chuan-nong 19, Line AIM6
*Yr*42	6A	*Aegilops neglecta*, Line 03M119-71A
*Yr*43	2BL	IDO377s=PI 591045, Lolo
*Yr*44	2BL	Zak-PI607839
*Yr*45	3DL	PI1811434
*Yr*46	4D	PI250413, RL6077=Thatcher*/PI250413
*Yr*47	5BS	AUS28183=V336
*Yr*48	5AL	UC1110(S)/PI60750 RIL 167 R
*Yr*49	3DS	Chuanmai 18
*Yr*50	4BL	*Thinopyrum intermedium*, Line CH2233
*Yr*51	4AL	AUS27854
*Yr*52	7BL	PI183527
*Yr*53	2BL	PI480148
*Yr*54	2DL	RIL GID6032209, RIL GID6032334
*Yr*55	2DL	Frelon AUS 38882
*Yr*56	2AS	AUS 91575, Wollaroi AUS 99174
*Yr*57	3BS	AUS 91463, AUS 27858
*Yr*58	3BL	Sonora W195 AUS 19292
*Yr*59	7BL	PI660061, Avocet S / PI178759 F4-158, PI178759

thirds of fully resistant cultivars. A modelling approach taking into account quantitative resistance shows that the proportion of fully resistant cultivars in a three-component mixture can

6- Breeding for rust resistance

6.1 Seedling vs. Adult plant resistance

Global devastating large-scale periodical epidemics of *Pst* occurrs across the world; the challenge ahead the breeders and pathologists is to control rusts and to maintain stable wheat production. Cereals protection is based on chemical control and genetic resistance. The deployment of resistance genes has been suggested to be the most economical and environmentally friendly measure to control the disease (de Vallavieille-Pope *et al*., 1990; Singh *et al*., 2004). Genetic control has advantages for environmental and economic reasons, particularly for the possible development of resistance to fungicides in the developing world with no evidence for rust resistance recorded worldwide (Oliver, 2014). Generally, two classes of genes are utilized for breeding for rust resistance. The first class is the R-genes; they are pathogen race-specific in their action, or also called all stage resistance or seedling stage resistance and effective at all plant growth stages; it is easily phenotyped in the glasshouse on seedlings and recognized by a diverse range of hypersensitive reactions as described in Roelfs *et al*. (1992). R genes are mostly functional from seedling to adult growth stages if the pathotypes does not change. In general, all stage resistance is race specific (Qayoum & Line; 1985). Seedling resistance is most common in wheat genotypes by single genes or simple combination of single genes. Due to selection pressure and the ability of the *Pst* pathogen to evolve rapidly and produce new strains, race-specific genes can be easily overcome and produce the "Boom and Bust" cycles of wheat production (MacDonald & Linde, 2002). The type of resistance that follows the gene for gene types are given the following terminology: race specific, seedling, qualitative, major gene, vertical, monogenic, complete, etc.

The second class is called <u>A</u>dult <u>P</u>lant <u>R</u>esistance genes (APR) that shows susceptibility in the seedling stage but is usually functional at the post-seedling stage, in contrast to most R genes, the

*Yr*60	4AL	Avocet*3//Lalbmono1B*4/Pavon, GID 5934039, Bahadur, GID 177343
*Yr*61	7AS	Pindong 34
*Yr*62	4BL	PI192252, PI660060 = Avocet S / PI192252 F4-103
*Yr*63	7BS	AUS27955
*Yr*64	1BS	PI660064 = Avocet S / PI331260
*Yr*65	1BS	AvS / PI480016 F7-12
*Yr*66	3DS	AGG91584WHEA = MSP4543.1, VL892 = AGG91586WHEA
*Yr*67	7BL	AGG91585WHEA = MSP4543.4 , C306, C591
*Yr*AvS	-	Avocet R, Avocet S
*Yr*H9020	2DS	H9020-1-6-8-3
*Yr*KK	2BS	Kenya Kuku

[a] RS, SDR, NRS, HTAP, TDSSR and APR correspond to race-specific resistance, seedling disease resistance, non-race specific resistance, high temperature adult plant resistance, temperature sensitive seedling disease resistance, and adult plant resistance, respectively.

[-] corresponds to the unknown type of resistance.

levels of resistance conferred by single APR genes are only partial and may allow considerable disease development. APR genes function mainly at the adult stage and is considered more robust in term of resistance.The accumulation of several APR genes (QTLs) can confer high protection level. Two main factors are considered in the APR: the nutritional status and the temperature. High temperature APR (HATP) are considered often as race non-specific and quantitatively inherited (Chen, 2005) and triggered when the average temperature exceeds 21°C

and prevent eventually the secondary infection events (Chen *et al*., 2014). The types of resistance that do not follow the GFG manner are given the following terminology: non-race specific, adult plant resistance, quantitative, minor gene, partial, horizontal, polygenic, incomplete, general, slow-rusting (Caldwell, 1968) and others.

From the catalogued *Yr* genes, 41 are considered as seedling resistance, and 14 confer APR genes. A majority of *Yr* genes originate from *T. aestivum*, but several are derived from related genera or species, including *Secale cereale* (*Yr9*), *Aegilops* spp. (*Yr8*, *Yr17*, *Yr37*, *Yr38*, *Yr40* and *Yr42*), *T. spelta* (*Yr5*), *T. dicoccoides* (*Yr15*, *Yr35* and *Yr36*), *T. turgidum* (*Yr24*/*Yr26*, *Yr53*), *T. tauschii* (*Yr28*) and *Thinopyrum intermedium* (*Yr50*) (Chen, 2013). HTAP resistance genes have been characterized: Uauy *et al*. (2005) characterized *Yr36* from *Triticum turgidum* var. *dicoccoides* located on chromosome 6BS; Lin & Chen (2007) characterized *Yr39* in spring wheat cultivar 'Alpowa' located on 7BL and Ren *et al*. (2012) characterized *Yr52* on 7BL in spring wheat line 'PI 183527'.

6.2 Quantitative trait loci (QTL)

Quantitative resistance, like many other agronomic traits, is controlled with several genetic loci and this refers to quantitative trait loci (QTL). Individual QTL's have small or intermediate effects on reducing the disease but together, they can confer higher level of resistance to *Pst* following the Mendelian law (Singh & Rajaram, 1994). Quantitative resistance had been a point of debate from the seventies (Nelson, 1978; Parlevliet & Zadoks, 1977; Vanderplank, 1982). It is very essential to define the QTLs and their association with the molecular markers for a better understanding of the relation of resistance genes allowing description and localization of QTLs and genes within the genome through correlation between allelic variation and the phenotype (Agenbag *et al.*, 2012). Rosewarn *et al*. (2013) summarized that over than 140 QTLs have been identified with 49 chromosomal regions, described seven groups relating to the seven chromosomes groups and highlighted the most important regions where QTLs are identified. A large number of QTLs for resistance to *Pst* and their associated molecular markers have been reviewed by Boyd (2006), Chen (2005, 2013), Singh *et al*. (2004) and Wellings *et al*. (2012). QTLs for resistance to *Pst* have been mapped to all wheat chromosomes except 1D and 3A (Chen, 2005, 2013; Christopher *et al*., 2013; Powell *et al.*, 2013; Vazquez *et al*., 2012; Lu *et al*., 2009; Mallard *et al*., 2005; Imtiaz *et al*., 2004; Suenaga *et al*., 2003).

Some cases of race specificity have been described for QTLs. An example is the race-specificity between the new *Pst* races in Europe and QTLs in long-term effective adult plant resistance in wheat. Some single-QTL lines would be more susceptible and others more resistant to the new races (Sørensen *et al*., 2014).

6.3 Durable rust resistance

Durable and long lasting rust resistance in crucial in wheat breeding. Following Johnson‘s definition (1981), he stated “durable resistance that has remained effective in a cultivar during its widespread cultivation for a long sequence of generations or period of time in an environment favorable to a disease or pest. Vernacularly, the term “durable” was used in the reference to describe lasting resistance to diseases and pests. Durable resistance remains the debate of competing views of both mechanism of resistance (e.g., horizontal versus vertical resistance) and resistance deployment strategies (e.g., pyramids versus mixtures) (Mundt, 2014).

It is easy to breed resistant varieties with single large R gene for the dominance or partially dominance. Successful breeding for minor genes confronts many obstacles: 1) small to intermediate effects of individual with minor genes 2) dispersed presence of minor genes in different varieties or germplasm, 3) field selection environment lacking uniform and high disease pressure, 4) small population sizes in various generations, 5) necessity of pyramiding 3-5 genes to achieve adequate to high resistance levels, 6) presence of R-genes in parents used in crossing programs, 7) difficulty in distinguishing small effect R-genes from partial resistance genes especially for resistance to yellow rust, 8) higher genotype x environment interaction on the expression and effectiveness of partial resistance genes, and 9) slow progress in identifying linked molecular markers requiring a long-term commitment (Singh, 2012). Both Race-specific and non-race specific genes offer opportunities to achieve durable control of wheat rusts. CIMMYT approach of combination of 4-5 minor genes for the rusts tends to maintain low and stable final disease severities in field trials when experienced with aggressive races (Singh et *al*., 2012). More researches are conducted on non-race-specific resistance genes and identification of tightly linked molecular markers. In the highlight of novel technologies, such as genomic selection, gene pyramiding of multiple small minor genes can be used. Along field phenotyping is an essential step for achieving a strong breeding programs for durable resistance.

The literature review highlighted that pathogens are evolving and variation in both host and environmental changes. Pathogens need to migrate, tolerate, or to adapt constantly. The role of host and climate on pathogen population structure would reflect on the pathogen adaptation capacity to these two factors (McDonald & Linde, 2002). This adaptation is dependent on the population innate capacity to evolve which is linked to the level of diversity and recombination capacity of the pathogen population (Taylor *et al*., 1999). The recent invasion in yellow rust population and recurrent epidemics could be due to various factors which included monoculture of cultivars having limited number of resistance genes, adaptation of *Pst* fungus to warmer temperatures, genetic recombination in location where the alternate host is present.

The Fertile Crescent, known for the origin area of wheat 11000 years ago, was an area of emerging of repetitive epidemics of wheat yellow rust (Bahri *et al.*, 2009, Yahyaoui *et al*., 2002). Up till now, there were no studies assessing resistance of landraces to yellow rust in Lebanon and Syria. Little is known about probable yellow rust resistance in Lebanese and Syrian germplasm. The region lacks the information about effective yellow rust resistance genes toward the prevalent pathotypes carrying possibly many virulences. Many studies reported the invasion of PstS1/S2 strains across the six continents and described population structure of *Pst* populations. Over the last decade, contradictory results have been found: high diversity has been reported in Asian populations although only clonal populations were described so-far (Ali *et al.*, 2014b) and epidemics have occurred in regions considered up to now to be too hot for yellow rust (Hovmøller *et al.*, 2011; Wellings 2011). Furthermore, this pathogen was also recently characterized by worldwide emergence of aggressive strains (Hovmøller *et al.*, 2011) and several new invasive strains from exotic origin were discovered in Europe since 2011 (Sørensen *et al.*, 2014, Hovmøller *et al.*, 2015). A detailed population genetic structure of *Pst* in Lebanon and Syria remained unknown. The question addressed in the present time with the discovery of *Berberis* sp. as alternate host for *Pst*, is how is the Middle East region population structure, the presence of the recombination signature, the differentiation of Lebanese and Syrian population with the presence of geographic mountainous barrier between Lebanon and Syria and differentiation in host preference for each country (Lebanon is a Durum wheat grower and Syria a bread wheat grower). Moreover, little is known about virulence and pathotype structure in Syria and Lebanon. So it is interesting to highlight on genetic diversity of *Pst* populations in Lebanon and Syria following a previous study conducted on limited number of isolates by Bahri *et al*. (2009).

The role of the climate adaptation is further questioned by the recent expansion of *Pst* epidemics to the areas previously considered as too hot for *Pst* (South of the United States, West Australia, South Africa, Southern Europe), due to the recent spread of two high-temperature-adapted strains (Hovmøller *et al.*, 2008). These recent spread pathotypes reported in the United States since 2000 were more aggressive than old ones at high temperature (Milus *et al*., 2006). One of these pathotypes was also detected in West Australia (Wellings, 2007; 2011), Europe, and North Africa (Bahri *et al*., 2009), demonstrating the dispersal potential of this species. Other *Pst* strains originating from Mediterranean area, were observed in South of France and were also found to be adapted to warm temperature (Mboup *et al.*, 2012). Since temperature adaptation had already been observed in some French isolates of this fungus, it is interesting to test which Mediterranean fungal isolates perform better under warm and cool experimental penetration and incubation temperatures.

In this PhD thesis, I attempt to understand the role of host and climate on population structure of *Puccinia striiformis* f. sp. *tritici* in the Middle East, to have clearer understanding of *Pst* interaction with host resistance and temperature. I have conducted a study organized in three chapters:

- Resistance gene diversity for yellow rust in wheat elite lines, commercial varieties, and landraces from Lebanon and Syria
- Genetic and pathotype diversity in the *Puccinia striiformis* f. sp. *tritici* populations from Lebanon and Syria

- Effect of the temperature on aggressiveness components, infection efficiency and latency period, on *Puccinia striiformis* f. sp. *tritici* in the Middle East, by comparing *Pst* isolates collected from warm and cold locations.

CHAPTER 1

Resistance gene diversity for stripe rust in wheat elite lines, commercial varieties and landraces from Lebanon and Syria

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Keywords: Stripe (yellow) rust, wheat, gene postulation, seedling resistance

Abstract: Stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is the major threat to wheat production in Central, West Asia and North Africa (CWANA). Despite the availability of effective fungicides, host resistance has remained the most economical, effective and ecologically sustainable method for disease control. Understanding the genetic diversity of resistance to *Pst* is a key element in breeding for durable rust resistance. Little is known about the genetic structure of resistance to *Pst* in breeding germplasm and commercial wheat varieties grown in CWANA. Multipathotype tests on 87 bread wheat elite lines from the spring wheat breeding program at the International Center for Agricultural Research in the Dry Areas (ICARDA), 23 Lebanese bread and durum wheat varieties, and 28 Lebanese landraces were carried out using 11 French *Pst* pathotypes able to discriminate low and high infection types for *Yr1*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr25*, *Yr27*, and *Yr32*. All these genes except *Yr32* were postulated singly in Lebanese bread and durum wheat varieties, landraces and ICARDA elite lines. Combinations of two *Yr* genes, *Yr6*+*Yr9*, *Yr6*+*Yr17*, *Yr7*+*Yr1*, *Yr7*+*Yr4*, *Yr9*+*Yr1*, *Yr9*+*Yr3*, and *Yr9*+*Yr4* were common in ICARDA elite lines. *Yr3*, *Yr6*, *Yr7*, *Yr17* and *Yr27* were the most common postulated genes in ICARDA elite lines, in Lebanese bread and durum wheat varieties, and Lebanese landraces. Resistance diversity was found in the landraces varying from partial to complete resistant seedlings among susceptible landraces to the 11 pathotypes. Three landraces types had high proportions of plants resistant to the *Pst* pathotype 239E175*V*17, which carries many virulences.

The present study showed the presence of effective seedling resistance genes and gave some insights into the prevalence of *Yr*-genes in tested wheat genotypes, which can be very useful in development of resistant genotypes and control strategies against *Pst*. More detailed study is required for the adult plant resistance especially for the elite lines.

Introduction

In a world facing global climate change and subsequent food insecurity, feeding the ever-growing population through sustainable agricultural practices is a global challenge. By 2030 the demand for wheat (*Triticum aestivum* L.) is estimated to increase by 40% (House of Commons Environment, Food and Rural Affairs Committee 2009; http://www.parliament.uk). Either directly or indirectly through livestock feed, the staple crops rice, maize and wheat provide a substantial proportion of the caloric intake for the human population (http://faostat.fao.org).

Historically wheat production in many countries is limited by biotic stresses of which the three wheat rusts, including the stripe rust, represent a global threat. Stripe (yellow) rust of wheat, caused by the biotrophic fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a common disease of worldwide economic importance (de Vallavieille-Pope et al. 2012; Singh et al. 2004). In most wheat producing areas, yield losses caused by stripe rust range from 10-70% (Chen 2005), this variation depending on initial infection time, host density, susceptibility and nutritional status, disease development, and duration of the epidemic. During the last 40 years, there have been five major stripe rust epidemics in the CWANA region in 1973, 1978, 1995, 2005 and 2010. The two most recent epidemics were due to the successive emergence of *Pst* pathotypes presenting new virulences for widely used *Yr* genes *Yr9* and *Yr27* (Yahyaoui et al. 2002, Duveiller et al. 2007, Hodson and Nazari 2010, Morgounov et al. 2012, Sharma-Poudyal et al. 2013). According to Hovmøller et al. (2011), the aggressive strain PstS2, with virulence on *Yr2*, *6*, *7*, *8*, *9*, *25*, and *Yr27* was observed in high frequencies in the Red Sea area, East Africa and in Western and Central Asia between 2003 and 2008 whereas the first report of this strain in North America was in 2000 (Milus et al. 2009). This strain was present in 50% of the virulence profiles of isolates surveyed in Syria in 2011 (El Amil et al., unpublished data).

This damaging fungus causes losses in both yield and quality by reducing tillering and causing shrivelled grains (Roelfs et al. 1992) unless it is controlled by resistant cultivars or timely fungicide applications (Hau and de Vallavieille-Pope 2006). The deployment and use of resistant cultivars is the most economical and environmentally friendly measure to control the disease (Pink 2002; Singh et al. 2004; Pathan and Park 2007). An effective deployment of resistance genes for the management of stripe rust in wheat requires knowledge about the resistance status and the diversity of resistance genes in available cultivars (Kolmer 2003).

Furthermore, knowledge of the prevalent pathotypes is crucial. Nonetheless, because new virulence types of pathogens like *Pst* can arise frequently, thereby compromising the durability of resistance (McDonald and Linde 2002) it is important to deploy strategies to improve the durability of resistance. These include i) deploying new resistance genes in a controlled manner and over a restricted geographic scale, ii) combining several resistance genes within a single cultivar to slow the emergence of pathotypes that can infect them, and iii) combining race-specific resistance with non race-specific or partial resistance within a single cultivar. These approaches require a good knowledge of the resistance genes present in the breeding germplasm and commercial cultivars. Therefore, it is important to identify the resistance genes from different cultivars since some may have resistance genes in common even if they originated from genetically different sources. This will prevent the release of mega-cultivars that contain the same resistance genes or profiles (Statler 1984).

Gene postulation is based on the gene-for-gene relationship (Flor 1971) and involves the postulation of genetically characterized race-specific genes for resistance in a cultivar, based on that cultivar's reaction after being confronted with an array of pathotypes with diverse combinations of avirulence and virulence genes. It can identify the probable race-specific rust resistance genes (*Yr*) harboured in a large group of wheat lines. This method has been traditionally used for all three rust diseases (Roelfs and McVey 1979; Perwaiz and Johnson 1986; Dubin et al. 1989; Singh and Rajaram 1991). Using gene postulation procedure, stripe rust resistance genes were postulated in wild emmer wheat derivatives and advanced wheat lines from Nepal (Sharma et al. 1995), in French wheat lines (Robert et al. 2000), in Danish wheat cultivars (Hovmøller 2007), in Chinese wheat cultivars and advanced lines (Xia et al. 2007), and in Ethiopian bread wheat cultivars (Dawit et al. 2012).

Landraces were the principal focus of agricultural production until the end of the nineteenth century with the arrival of formal plant breeding (Harlan 1975). According to Camacho Villa

et al. (2005), “a landrace is a dynamic population(s) of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems”. Elite cereal cultivars are derived from a relatively narrow germplasm pool and are predominantly well adapted to high input agriculture. A distinction is made between landraces and modern, or so called elite lines, the latter being the result of formal crop breeding programs (Newton et al. 2010). Landraces might be a good reservoir of non race-specific or partial resistances that may confer durability when combined with major resistance gene commonly exploited in modern cultivars. Landraces could expand the narrow genetic basis of elite lines, introducing adaptation to local edaphic and climatic conditions as well as tolerance and resistance to pests and diseases (Beharav et al. 1997). Zhang (1995) demonstrated that nine Chinese landraces expressed slow rusting or quantitative resistance to stripe rust. Since Lebanon is located in the Near East Fertile Crescent, the most diverse area for wheat and its wild relatives (Harlan and Zohary 1966), Lebanese landraces are also likely to be promising sources of novel resistance genes with both major and partial effects. Therefore identifying seedling resistance genes in Lebanese landraces is an important first step to further wheat improvement, which has not yet been initiated at CWANA region.

The resistance genes to wheat stripe rust of ICARDA elite breeding lines, Lebanese cultivars and Lebanese landraces remain largely unknown. Therefore, this study was designed to provide detailed information about specific resistance to wheat stripe rust detectable at the seedling stage of 87 bread wheat elite lines from the spring wheat breeding program at ICARDA, 23 Lebanese bread and durum varieties, and 28 Lebanese landraces. Gene postulation was carried using an array of 11 *Pst* pathotypes that differentiate low and high infection types for *Yr1*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr25*, *Yr27*, *Yr32*, *YrSD*, *YrSu* and *YrSP*. Adult plant resistance of some ICARDA lines was also evaluated.

Materials and Methods

Pathogen Materials

Virulence combinations and pathotype codes of *Pst* isolates used for resistance gene postulation were determined using the European and World sets of 15 differential varieties (Johnson et al., 1972), to which were added 6 wheat lines with known resistance profiles:

Table 1. Pathotype code, name and avirulence/virulence formula of 11 French *Puccinia striiformis* f. sp. *tritici* pathotypes used in postulation of stripe rust resistance genes in Syrian and Lebanese wheat genotypes

Pathotype code	**Pathotype nomenclature** [1]	**Avirulence/ virulence formula**
A	6E 16	1, 3, 4, 5, 9, 10, 15, 17, 24, 25, 26, 27, 32, SD, Su, ND, SP/ 2, 6, 7, 8
B	6E 16 *V*27	1, 3, 4, 5, 10, 15, 17, 24, 26, 32, SD, Su, ND, SP/ 2, 6, 7, 9, 8, 25, 27
C	43E 138	4, 5, 6, 7, 8, 9, 10, 15, 17, 24, 26, 27, 32, Su, SP/ 1, 2, 3, 7, 25, SD, ND
D	45E 140	4, 5, 7, 8, 9, 10, 15, 17, 24, 26, 27, 32, Su, SP/ 1, 2, 3, 6, 25, SD, ND
E	106E 139	1, 5, 6, 8, 9, 10, 15, 17, 24, 26, 27, 32, SP/ 2, 3, 4, 7, 25, SD, Su, ND
F	169E 136*V*17	4, 5, 6, 7, 8, 10, 15, 24, 26, 27, 32, Su, SP/ 1, 2, 3, 9, 17, 25, SD, ND
G	232E 137	1, 5, 6, 7, 8, 10, 15, 17, 24, 26, 27, 32, SP/ 2, 3, 4, 9, 25, SD, Su, ND
H	237E 141	5, 7, 8, 10, 15, 17, 24, 26, 27, 32, SP/ 1, 2, 3, 4, 6, 9, 25, SD, Su, ND
I	237E 141*V*17	5, 7, 8, 10, 15, 24, 26, 27, 32, SP/ 1, 2, 3,, 4, 6, 9, 17, 25, SD, Su, ND
J	237E 173*V*17	5, 7, 8, 10, 15, 24, 26, 27, SP/ 1, 2, 3, 4, 6, 9, 17, 25, 32, SD, Su, ND
K	239E 175*V*17	5, 8, 10, 15, 24, 26, 27/ 1, 2, 3, 4, 6, 7, 9, 17, 25, 32, SD, Su, ND, (SP)

[1] Pathotype nomenclature is based on Johnson et al. 1972.

YrSD, *YrSu*, *YrND* and *YrSP,* correspond for Strubes Dickkopf, Suwon 92xOmar, Nord Desprez and Spaldings Prolific, respectively.

Kalyansona (*Yr2*), Federation 4*/ Kavkaz (*Yr9*), Clement (*Yr9*+), VPM1 (*Yr17*+), TP981 (*Yr25*), Opata (*Yr27*+*Yr18*) and 13 Avocet Near Isogenic lines for *Yr1*, *Yr5*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr15*, *Yr24*, *Yr26*, *Yr27*, *Yr32*, *YrSP* (http://www.ars.usda.gov/SP2UserFiles/ad_hoc/36400500Resistancegenes/Yrgene.xls). Each differential line carries at least one race-specific resistance gene (*Yr*) expressed at the seedling stage.

Resistance genes were postulated at the seedling stage at INRA BIOGER station based on infection types (IT) using a set of 11 French pathotypes displaying complementary virulences (Table 1) (de Vallavieille-Pope et al. 1990; de Vallavieille-Pope et al. 2000; de Vallavieille-Pope et al. 2012). As most of these pathotypes present more than one avirulence factor, precise resistance gene combinations could not always be inferred.

All isolates belonging to the INRA-Grignon collection had been purified from single spores and stored in liquid nitrogen for various times before the beginning of this study. Spore multiplication was performed in a climate-controlled room. Spores of reference isolates were inoculated onto 7-day-old seedlings of the susceptible cultivar Victo, incubated in a dew chamber at 8°C for 16 h in the dark to ensure successful infection, and then transferred to a climate chamber (day: 16 h, 300 µmol m^2 s^{-1}, 17°C; night: 8 h, 14°C). High intensity light treatment was applied to the seedlings prior to inoculation for at least 8 h in order to maximize infection success (de Vallavieille-Pope et al. 2002). A week after inoculation, each pot was sealed in a cellophane bag to avoid cross-contamination. Eighteen days post-inoculation, uredospores were collected, dried in a desiccator filled with Silicagel at 4°C for 3 days, and stored in liquid nitrogen. After removal from storage, the uredospores were heat shocked (40°C for 10 min) before inoculation for *Yr*-gene postulation tests.

Host Materials

In total 138 genotypes comprising 87 bread wheat advanced lines from spring wheat breeding program at ICARDA, 23 (15 bread and 8 durum wheat cultivars) commonly grown in Lebanon and 28 Lebanese landraces (bread and durum wheat) were tested. Seed stocks for elite lines and landraces were obtained from ICARDA and LARI (Lebanese Agriculture Research Institute) germplasm, respectively. The landraces are known for diversity and heterogeneity, so landrace seeds were collected from different Lebanese sites to obtain a

broad genetic pool and then were purified at the Lebanese agricultural research institute for morphological traits before resistance gene tests.

Inoculation and scoring

All seeds were planted in square pots (7x7x8 cm) filled with standard peat soil. We planted 5 seeds of elite lines and varieties and 15 seeds of each landrace in each of two replicated pots placed in air-filtered cabinets in a glasshouse at temperatures between 15 and 25°C with a 16-h photoperiod extended with sodium vapour lamps. Inoculation was done as described previously. The experimentation was repeated two times.

Seedling infection types were recorded 15-17 days after inoculation using 0- 9 scale based on the presence of necrosis, chlorosis and the size and intensity of sporulation (McNeal et al. 1971) in which infection types 0 to 4 indicated varying levels of incompatibility (host resistance and pathogen avirulence) between host and pathogen, infection types (IT) 7-9 indicated compatible (host susceptibility and pathogen virulence) interactions and infection types 5 to 6 were considered intermediate reactions (Roelfs et al 1992). Resistance genes in the tested genotypes were postulated by comparing the low and high IT patterns produced by the pathotype set on these tested genotypes with those on differential wheat genotypes with known resistance genes. When a wheat variety had a similar low/high IT pattern to that of a differential line with known resistance profile the tested genotype was postulated to possess the same resistance gene(s). Low ITs can confirm the postulation of the *Yr* gene toward specific virulences. This method was applied successively on all *Yr* genes detectable with the array of 11 *Pst* pathotypes used for the study.

Adult plant stage resistance assessment

Of the ICARDA's elite lines tested at seedling stage, 47 lines were assessed for adult-plant resistance at Tal Hadya research station in Syria during 2009-2011 and Terbol research station in Lebanon during 2009 to 2013.

Thirty seeds of each genotype were planted in two 0.5 m rows in a field nursery by November of each year. The highly susceptible *cv*. Morocco, Seri-82 and Sham-8 were planted as spreader rows bordering the trial area, in all pathways, and every 10 wheat lines within the trial. The inoculum used for this study comprised the dominant isolate collected in the field during the previous year separately in both Syria and Lebanon. The prevalent inoculated pathotypes carried virulence for the genes *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27* and

Table 2. Resistance group, seedling infection types and postulated yellow rust resistance genes in 87 wheat elite lines from ICARDA against 11 *Puccinia striiformis* f. sp. *tritici* pathotypes.

Group	Entry No.	genotype	Pathotype code[a]											*Postulated Yr-gene/s*
			A	B	C	D	E	F	G	H	I	J	K	
1	**63**	Bush/Amigo T101 X Sakha 69//Shuha-5/3/Bocro-3	1	1	3	6	1	5	1	3	2	2	5	Resistant[b]
	64	Tracha'S'//CMH76-252/PVN'S'	1	2	3	6	1	5	1	3	2	2	4	Resistant
	65	Achtar*3//Kanz/KS85-8-4/3/Zemamra-5	1	1	2	2	1	2	1	2	2	2	3	Resistant
	66	Blass-1/4/CHAT'S'//KVZ/CGN/3/BAU'S'	1	1	2	2	1	4	1	2	2	2	4	Resistant
	67	Crow'S'/Bow'S' -1994/95//Asfoor-5	1	5	4	3	3	3	2	2	2	3	4	Resistant
	68	Saba/Flag-1	2	6	2	2	2	2	2	2	2	2	4	Resistant
	69	CBRD/Kauz//Weebill 1/3/Atena-1	1	2	3	4	1	6	1	2	2	2	5	Resistant
	70	Shuha-8/Ducula	1	1	2	2	1	3	1	2	2	2	4	Resistant
2	**71**	W3918A/JUP	8	7	8	8	9	8	8	8	8	8	8	Susceptible[c]
	72	Nesma*2/14-2//2*Safi-3	8	7	8	8	9	8	8	8	8	8	8	Susceptible
	73	Shuha-8/Ducula	8	6	8	8	8	8	8	8	8	6	8	Susceptible
		Chinese 166 (*Yr1*)[d]	1	1	9	9	1	8	1	9	9	9	9	*Yr1*
3		**Avocet *Yr*1/6*Avocet S**	2	1	8	8	2	8	1	8	8	8	9	*Yr1*
	1	Crow'S'/Bow'S' -3-1994/95//Tevee'S'/Tadinia	1	1	8	8	1	9	1	8	7	8	7	*Yr1*
	2	Tevee'S'/3/T.aestivum/SPRW'S'//CA8055/4/Pastor-2/5/Sunbri	1	1	8	8	1	9	1	8	8	8	8	*Yr1*
	3	Qafzah-2/Ferroug-2	1	2	8	8	1	9	2	8	8	8	8	*Yr1*
	4	Crow'S'/Bow'S' -1994/95//Asfoor-5	1	2	8	8	1	8	1	8	8	8	8	*Yr1*
	5	Ferroug-2/Potam*2KS811261-8//Zemamra-8	2	2	8	8	1	7	1	8	8	8	8	*Yr1*
		Vilmorin 23	2	3	8	8	9	8	8	9	9	9	9	*Yr3*
4	**6**	Bow #1/Fengkang15/3/HYS//DRC*2/7C	5	3	8	8	8	9	7	8	8	8	8	*Yr3*
5		**Hybrid 46**	2	1	1	2	9	2	8	9	9	9	8	*Yr4*
	7	SHA3/Seri//Yang87-142/3/2*Towpe	2	3	2	2	8	2	8	8	8	8	8	*Yr4*
	8	SHA3/Seri//Yang87-142/3/2*Towpe	2	2	2	2	8	2	8	6-7	8	8	8	*Yr4*
	9	ESWYT99#18/Arrihane	2	5	4	2	8	3	7	8	8	8	8	*Yr4*
6		**Avocet *Yr*6/6*Avocet S**	8	8	3	8	4	4	4	8	8	8	8	*Yr6*
		Heines Kolben	9	9	2	9	2	2	1	9	9	9	9	*Yr6, Yr2*
	10	Hamam-4/Angi-2	8	8	3	8	3	3	2	8	8	8	8	*Yr6*
	11	Hubara-16/2*Somama-3	7	8	2	8	2	2	3	8	6	8	8	*Yr6*
		Heines Peko	2	4	2	9	2	2	2	9	9	9	8	*Yr6, Yr+*
	12	Cham-4//Shuha'S'/3/SD 8036	2	2	2	9	2	2	2	2	2	7	8	*Yr6, Yr+*
	13	Aguilal/Flag-3	2	3	2	8	2	2	2	8	5	8	8	*Yr6, Yr+*
	14	MON'S'/ALD'S'//Towpe'S'	1	5	4	8	1	3	4	7	8	8	8	*Yr6, Yr+*
	15	Weebill-1/2*Qafzah-21	4	6	3	7	7	2	5	8	8	8	8	*Yr6, Yr+*
	16	Rebwah-12/Zemamra-8	2	1	2	2	1	3	1	8	8	8	8	*Yr6, Yr+*
7		**Avocet *Yr*7/6*Avocet S**	8	8	8	3	8	4	4	2	3	3	8	*Yr7*
		Lee	9	9	9	3	9	3	2	3	3	3	9	*Yr7, Yr+*
		Reichersberg 42	2	4	9	2	9	2	2	2	2	2	8	*Yr7, Yr+*
	17	Samar-8/Kauz'S'//Cham-4/Shuha'S'	1	2	1	1	1	1	2	2	2	2	8	*Yr7, Yr+*
	18	Shuha-4/6/GV/ALD'S'/5/ALD'S'/4/BB/GLL//CNO67/7C/3/KVZ/TI/7/PRL'S'/ Vee'S'/3/P106.19//Soty/	1	2	1	1	1	1	2	2	2	2	8	*Yr7, Yr+*
	19	Kauz//Kauz/Star	1	1	1	1	1	1	2	1	2	2	8	*Yr7, Yr+*
	20	Cham-4/Shuha'S'/6/2*Saker/5/RBS/Anza/3/KVZ/HYS//YMH/TOB/4/Bow'S'	1	1	1	1	1	1	2	1	2	2	8	*Yr7, Yr+*
	21	Cham-4/Shuha'S'/6/2*Saker/5/RBS/Anza/3/KVZ/HYS//YMH/TOB/4/Bow'S'	1	1	1	1	-	1	2	1	2	2	8	*Yr7, Yr+*

YrSD. The field was dusted in the evening with a spore-talc mixture (1 to 50) at the seedling, tillering, and flag leaf stages. The disease infection types were recorded according to Roelfs et al. (1992) and the modified Cobb scale was used for disease severity (Peterson et al., 1948).

Results

Seedling resistance (All-stage resistance) Seedling tests conducted on the 138 elite lines, varieties and landraces with eleven French *Pst* pathotypes allowed postulation of seedling stripe rust resistance genes either singly or in

combination (Tables 2, 3, and 4). The pathotypes used allowed the postulation of *Yr1*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr9*, *Yr17*, *Yr25*, *Yr27* and *Yr32*. Based on these postulations, the lines were placed in thirteen stripe rust resistance groups.

Resistance group 1, corresponding to genotypes resistant to all *Pst* pathotypes, included eight ICARDA bread wheat elite lines (Table 2) and three Lebanese durum wheat varieties (Table 3). These genotypes showed low to intermediate ITs to all tested *Pst* pathotypes, indicating that they possess a *Yr* gene or combination of *Yr* genes that had no corresponding virulence(s) in the 11 *Pst* test pathotypes.

Resistance group 2 corresponded to the genotypes susceptible to all 11 *Pst* test pathotypes, therefore harbouring no *Yr* genes that had not been overcome by all 11 *Pst* pathotypes. Three elite lines (Table 2), one Lebanese variety (Table 3) and 21 Lebanese landraces (14 populations of Salamouni, three populations of Abou Shwereb, two populations of Ukrainian, and one population of each Bekaii and Haurani) showed high ITs ranging from 8 to 9 when confronted with all *Pst* pathotypes (Table 4).

Resistance group 3 was postulated to harbour the resistance gene *Yr1*, as characterised by the tester genotypes Chinese 166 and the Avocet *Yr1* known to have the resistance gene *Yr1*. Plants belonging to this group displayed high ITs of 7 to 9 when confronted with the seven *Pst* pathotypes virulent to *Yr1* and low infection types of 1 to 3 against the four *Pst* pathotypes avirulent to *Yr1*, and were found only in the ICARDA elite lines.

Resistance group 4 was postulated to harbour the resistance gene *Yr3* as characterised by the tester genotype Vilmorin 23 and the nine *Pst* pathotypes virulent for*Yr3* and the two *Pst*

	22	Florkwa-2/Asfoor-5	1	2	1	1	1	1	2	2	2	2	8	*Yr7, Yr+*
	23	Ferroug-2/Potam*2KS811261-8//Zemamra-8	1	2	1	1	1	1	3	2	2	2	8	*Yr7, Yr+*
	24	Hubara-15/Zemamra-8	1	1	1	1	1	3	1	2	3	5	8	*Yr7, Yr+*
	25	Cham-4/Shuha'S'/6/2*Saker/5/RBS/Anza/3/KVZ/HYS//YMH/TOB/4/Bow'S'	1	2	1	1	1	1	2	2	2	2	8	*Yr7, Yr+*
	26	Shuha-5/Asfoor-1	1	1	1	1	1	1	2	1	2	2	8	*Yr7, Yr+*
	27	Achta*3//Kanz/KS85-8-4/3/Lakta-8/4/Zemamra-1	2	2	2	1	2	1	2	2	2	2	8	*Yr7, Yr+*
	28	Cham-4/Shuha'S'/6/2*Saker/5/RBS/Anza/3/KVZ/HYS//YMH/TOB/4/Bow'S'	1	1	1	1	1	1	1	1	1	1	8	*Yr7, Yr+*
	29	Turaco/CHIL/6/Seri 82/5/ALD'S'/4/BB/GLL//CNO67/7C/3/KVZ/TI	1	1	1	1	1	1	2	1	2	2	8	*Yr7, Yr+*
	30	Achtar/INRA 1764	2	8	2	5	2	3	2	2	4	6	8	*Yr7, Yr+*
	31	Achtar/INRA 1764	2	8	3	3	2	4	3	4	3	6	8	*Yr7, Yr+*
8		**Avocet *Yr9*/6*Avocet S**	2	8	2	2	2	8	8	8	8	8	8	*Yr9*
		Clement	1	5-6	2	1	2	8	8	8	8	8	9	*Yr9*
		Federation 4*/ Kavkaz	1	9	2	1	1	8	8	9	9	9	9	*Yr9*
	32	MON'S'/ALD'S'//Aldan'S'/IAS58/3/Safi-1/4/Zemamra-1	3	8	5	1	1	8	8	7	8	8	8	*Yr9*
	33	Shuha'S'/Tamega//Bow#1/Fengkang15	2	8	2	2	2	8	9	-	8	8	8	*Yr9*
	34	Clement/ALD'S'//Zarzour/5/AU//KAL/BB/3/BON/4/KVZ//CNO/PJ62 (Sandall 3)	1	8	2	2	2	8	9	-	8	8	8	*Yr9*
	35	ICARDA-SRRL-5	2	8	2	2	2	8	8	-	8	8	8	*Yr9*
9		**VPM1**	2	3	2	2	2	8	2	2	9	9	9	*Yr17*
	36	F5 Derived Kenya (D.H #100) F2	1	1	1	2	1	7	1	2	8	8	8	*Yr17*
	37	Clement/ALD'S'//Zarzor/5/AU//KAL/BB/3/BON/4/KVZ//CNO/PJ62 (Sandall 5)	2	3	2	2	2	9	2	2	8	8	8	*Yr17*
	38	NS732/HER//Milan/SHA7	1	4	2	2	1	8	2	2	8	8	8	*Yr17*
	39	Shuha-8//Vee'S'/Saker'S'	1	2	5	2	1	5	2	3	8	8	8	*Yr17+*
10		**TP981**	2	8	9	9	9	8	8	9	9	9	9	*Yr25*
	40	Tevee'S'//Kauz'S'/3/Fow'S'//NS732/HER	1	8	8	9	9	9	8	8	8	8	8	*Yr25*
11		**Avocet *Yr27*/6*Avocet S**	5	8	3	2	3	3	2	3	2	2	3	*Yr27*
		Opata	5	8	3	2	3	3	2	3	2	2	3	*Yr27*
	41	Kauz = JUP/BJY//URES	1	8	1	1	1	2	2	2	2	2	5	*Yr27*
	42	Inqualab 91/Flag-2	4	8	2	3	2	2	2	2	3	3	4	*Yr27*
	43	Bow#1/Fengkang15/3/HYS//DRC*2/7C	2	8	2	3	2	2	2	2	2	2	2	*Yr27*
	44	NS732/HER//SD8036/3/Saada	2	7	2	2	2	2	5	2	2	2	2	*Yr27*
	45	CBME4SA#4/Fow-2	1	8	1	1	1	2	2	2	2	2	3	*Yr27*
	46	Saker/5/RBS/Anza/3/KVZ/HYS//YMH/TOB/4/Bow'S'/6/PEWIT 3/7/Atena-1	2	8	4	2	5	3	2	2	2	2	3	*Yr27*
	47	NS732/HER*2//Saada	2	8	2	2	3	2	2	2	2	2	2	*Yr27*
	48	Tevee'S'/Kauz'S'//Attila-5/6/GV/ALD'S'/5/ALD'S'/4/BB/GLL//CNO67/7C/3/KVZ/TI	1	8	1	1	1	2	4	2	2	3	4	*Yr27*
12	**49**	GV/ALD'S'/5/ALD'S'/4/BB/G11//CNO67/7C/3/KVZ/TI/6/2*Towpe	4	6-7	3	4	5	2	5	8	8	8	8	*Yr6 + Yr9*
	50	PSN'S'/Bow'S'//Kauz'S'/3/Safi-1	1	8	1	1	1	5	4	8	8	8	8	*Yr6 + Yr9*
	51	DVERD-2/*Aegilops squarrosa* (214)//2*ESDA/3/NS732/HER	1	3	2	1	1	4	4	2	8	7	8	*Yr6 + Yr17*
	52	Hubara-3/Angi-2//Somama-3	1	1	2	1	1	2	1	4	7	8	8	*Yr6 + Yr17*
	53	Giza-164/Yebroud-1//Booma-2	2	1	7	3	2	2	1	2	3	2	8	*Yr7 + Yr1*
	54	Ghurab-2//Turaco/CHIL	2	2	2	1	7	1	2	2	2	2	8	*Yr7 + Yr4*
	55	Samar-8/Kauz'S'//Cham-4/Shuha'S'	2	2	1	1	9	1	2	2	2	2	8	*Yr7 + Yr4*
	56	VAN'S'/3/CNDR'S'/ANA//CNDR'S'/MUS'S'/4/Tevee-5	2	2	2	1	8	2	2	2	2	2	8	*Yr7 + Yr4*
	57	ACSAD 529/Karawan'S'//Somama-3	1	1	3	2	1	9	1	8	8	8	8	*Yr9 + Yr1*
	58	Hubara-5/3/SHA3/Seri//SHA4/Lira	1	2	2	3	2	8	8	9	8	8	8	*Yr9 +Yr3*
	59	Qafzah-33/Florkwa-2	1	4	2	2	1	3	8	-	8	8	8	*Yr9 + Yr4*
	60	Samar-12/Dollarbird	1	1	1	1	2	1	8	-	8	8	8	*Yr9 + Yr4*
	61	Milan/SHA7//Potam*3KS811261-5	1	4	1	1	2	3	8	-	7	8	8	*Yr9 + Yr4*

pathotypes avirulent for *Yr3*. One ICARDA elite line, one Lebanese variety, and two Lebanese landraces belonged to this group (Tables 2, 3 and 4).Resistance group 5 was postulated to harbour the resistance gene *Yr4* as characterised by the tester genotype Hybrid 46. Plants belonging to this group showed high ITs (7-9) when confronted with the six pathotypes virulent to *Yr4* and low to intermediate ITs of 2 to 5 when confronted with the five pathotypes avirulent to *Yr4*. Three elite lines and the bread wheat landrace Nessr belonged to this group (Tables 2 and 4).

Resistance group 6 was postulated to harbour the resistance gene *Yr6*, either alone, as characterised by the tester line Avocet *Yr6* or together with additional *Yr* genes like the tester genotype Heines Peko. The lines placed in this group showed high ITs of 7-8 when

confronted with at least some of the seven pathotypes virulent to *Yr6* and low ITs (1-3) when confronted with the 4 pathotypes avirulent to *Yr6* (Table 2). Five bread wheat elite lines were postulated to carry *Yr6* plus additional *Yr* genes.

Resistance group 7 was postulated to harbour the resistance gene *Yr7*, either alone, as characterised by the tester line Avocet *Yr7*, and observed for one Lebanese bread wheat variety or together with additional *Yr* genes like the tester genotype Riechersberg 42, as was found for 15 elite lines and three Lebanese bread wheat varieties.

Resistance group 8 was postulated to harbour the resistance gene *Yr9*, showing similar infection phenotypes as the testers Avocet *Yr9* and Clement. This group included four bread wheat elite lines and one Lebanese durum wheat variety (Tables 2 and 3).

Resistance group 9 was postulated to harbour the resistance gene *Yr17*, like the tester VPM 1, and included four elite lines (Table 2).

Resistance group 10 was postulated to carry *Yr25* like the tester line TP981, and included one elite line, one Lebanese bread wheat variety and two landraces (Tables 2, 3 and 4).

Resistance group 11 was postulated to carry *Yr27*, like the tester genotypes Avocet *Yr27* and Opata, and included eight elite lines and four Lebanese bread wheat varieties (Tables 2 and 3).

Resistance group 12 was postulated to carry two *Yr* genes: Some members of this group, namely two Lebanese varieties (one bread wheat and one durum wheat) showed high ITs only to pathotypes virulent on both *Yr*6 and *Yr*7 and low ITs in the absence of virulence for *Yr6* and/or *Yr7* or both genes were postulated to carry *Yr6*+*Yr7* (Table 3). The Lebanese

62	Andalieb-5//Tevee-1/Shuha-6	1	2	1	1	1	3	8	-	8	8	8	*Yr9 + Yr4*
74	Utique 96/Flag-1	4	7	3	8	9	5	9	8	8	8	8	Ni[f]
13													
75	T.aestivum/SPRW'S'//CA8055/3/Bacanora86	1	7	1	1	1	5	7	3	8	8	4	Ni
76	Fow'S'//NS732/HER/3/Cham-6//Ghurab'S'	2	8	3	6	9	5	8	3	8	8	2	Ni
77	Fow-2/SD8036//SafiI-3/3/NS732/HER//Kauz'S'	3	8	5	8	8	8	8	3	8	5	2	Ni
78	NS732/HER//Arrihane/3/PGO/Seri//BAU	1	8	1	8	-[e]	2	3	2	2	2	4	Ni
79	IZAZ-2//Tevee'S'/Shuha'S'	2	2	8	1	2	9	2	8	8	8	8	Ni
80	PFAU/Milan//Cham-4	5	3	3	2	4	8	2	4	3	2	8	Ni
81	Sakha73/5/IAS 58/4/KAL/BB//CJ'S'/3/ALD'S'/6/Goumria-12	2	2	3	7	8	8	8	8	4	3	3	Ni
82	Stat'S'/Florkwa-2	7	8	4	3	8	5	8	6	2	3	8	Ni
83	Tevee'S'/3/T.aestivum/SPRW'S'//CA8055/4/Pastor-2/5/Sunbri	1	1	8	8	1	8	1	8	4	8	8	Ni
84	Qafzah-5/2*Regrag-1	2	2	8	8	1	1	6	6	2	8	8	Ni
85	HD2206/Hork'S'/3/2*NS732/HER//Kauz'S'	4	6	5	8	7	9	8	7	7	8	8	Ni
86	Girwill-13/2*Pastor-2	1	8	3	2	2	4	8	8	8	8	8	Ni
87	Shuha-4//NS732/HER	6	1	8	8	1	9	1	8	8	8	8	Ni

[a]A= 6E16, B= 6E16V27, C= 43E138, D= 45E140, E= 106E139, F= 169E136V17, G= 232E137, H= 237E141, I= 237E141V17, J= 237E173V17, K=239E175V17. Pathotypes were coded according to Johnson et al. (1972). The virulences and avirulences tested were Vr1,2,3,4,6,7,8,9,17,25, 27,32,SD,SP,Su. Scoring was done according to McNeal et al.(1971); Infection types IT0= No visible uredia, IT1= Necrotic flecks, IT2= Necrotic areas without sporulation, IT3-4= Necrotic and chlorotic areas with restricted sporulation, IT5-6= Moderate sporulation with necrosis and chlorosis, IT7-8= Sporulation with chlorosis, IT9= Abundant sporulation without chlorosis.

[b] Resistant to all *Pst* pathotypes used

[c] Susceptible to all *Pst* pathotypes used

[d] The entries in bold corresponds to the infection type profiles of the tester lines when confronted with the array of 11 *Puccinia striiformis* f. sp. *tritici* pathotypes

[e] corresponds to missing data

[f] Ni corresponds to non-identified resistance genes

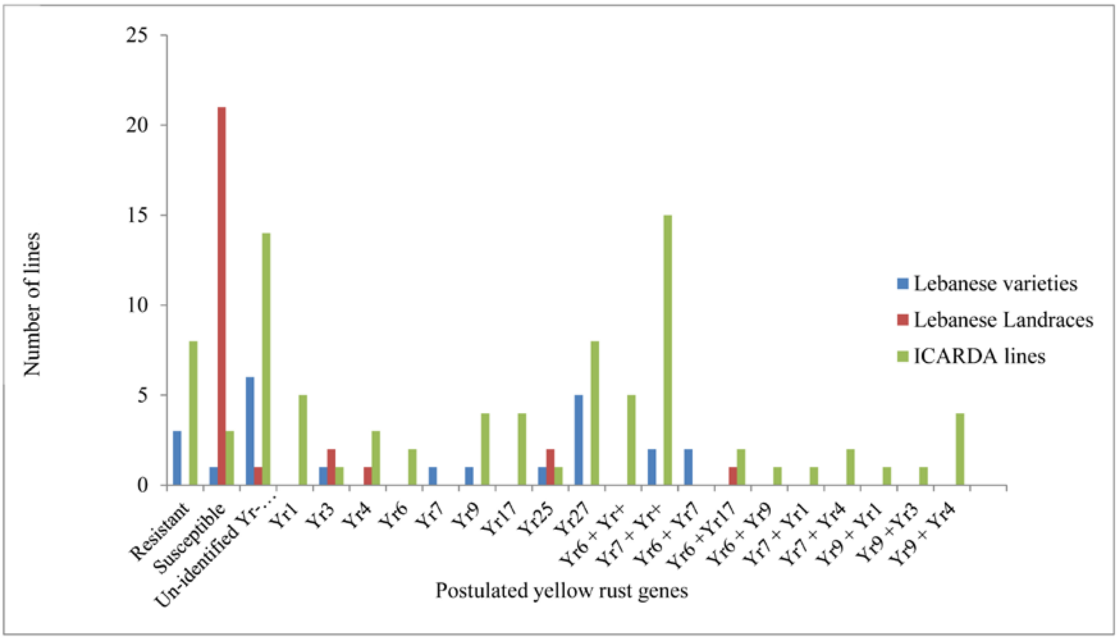

Figure 1. Frequency of postulated *Yr* genes in ICARDA lines, Lebanese varieties, and Lebanese landraces.

landrace Naama and two elite lines were postulated to carry *Yr6+Yr17* giving low ITs in the absence of one or both virulences and high IT only to pathotypes possessing both virulences for *Yr*6 and *Yr17*. Other members of this group, namely two elite lines from ICARDA, were postulated to carry *Yr6+Yr9*, showing intermediate reaction (IT 4-5) to pathotypes avirulent on *Yr6* and *Yr9* and high IT to the pathotypes virulent on *Yr6* and *Yr9.* Six elite lines had one resistance gene in addition to *Yr9, i.e., Yr1*, *Yr3* and *Yr4* in one, one, and four lines, respectively. One elite line showed high IT to pathotypes virulent to *Yr1* and *Yr7* and low IT to the avirulent pathotypes to the mentioned genes together, which led to postulate *Yr1+Yr7.*

Three elite lines were postulated to have *Yr7+Yr4* showing high ITs to the pathotypes virulent to *Yr7+Yr4* and low ITs to the pathotypes avirulent to *Yr7+Yr4.*

Resistance group 13, showing unexplained or uncharacterized resistances that could not be attributed to known resistance genes observed in other lines, included 14 elite lines (Table 2), one Lebanese bread wheat variety and five Lebanese durum wheat varieties (Table 3), and one Lebanese durum wheat landrace (Table 4).
None of the tested lines was postulated to carry *Yr32.*

Except for three elite lines and one Lebanese variety being fully susceptible at the seedling stage against the array of pathotypes and 15 elite lines and seven Lebanese varieties having un-identified *Yr* genes, all ICARDA elite lines and Lebanese varieties carried at least one *Yr* gene, whereas 75% of landraces were susceptible to all pathotypes. Nine tested resistance genes (*Yr1*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr9*, *Yr17*, *Yr25*, and *Yr27*) were postulated singly in ICARDA elite lines, *Yr7, Yr9, Yr25, Y27* were detected in Lebanese varieties and only *Yr3, Yr4, Yr25* were detected in landraces. Gene combinations of two *Yr* genes were found in 40% of ICARDA elite lines: *Yr6+Yr9*, *Yr6+Yr17*, *Yr7+Yr1*, *Yr7+Yr4*, *Yr9+Yr1*, *Yr9+Yr3*, and *Yr9+Yr4*. The *Yr6+Yr7* combination was found in two Lebanese varieties and *Yr6+Yr17* in one landrace only. The postulated *Yr* genes, either singly or in combination, were more frequent in Lebanese varieties than in landraces. ICARDA elite lines accumulated more *Yr* genes than the Lebanese varieties and landraces (Fig. 1)

Evaluation of adult plant resistance

Table 3. Resistance group, infection types and postulated yellow rust resistance genes at the seedling stage towards 11 *Puccinia striiformis* f. sp. *tritici* pathotypes for 23 Lebanese varieties.

Resistance group	Wheat line	Pathotype code[a]											Postulated *Yr*-genes
		A	B	C	D	E	F	G	H	I	J	K	
1	Waha	1	2	2	2	2	1	1	2	1	2	1	Resistant[b]
	Stork	1	3	2	2	3	4	4	2	2	2	1	Resistant
	Azeghar	3	3	2	2	2	1	2	2	2	2	2	Resistant
2	Super X	8	8	8	8	-[c]	9	-	9	8	8	9	Susceptible[d]
4	**Vilmorin 23**[f]	2	3	8	8	9	8	8	9	9	9	9	
	Genessi	3	3	7	8	6-7	9	8	8	8	8	8	*Yr*3
7	**Avocet *Yr*7/6*Avocet S**	8	8	8	3	8	4	4	2	3	3	8	*Yr7*
	Lee	9	9	9	3	9	3	2	3	3	3	9	*Yr7, Yr+*
	Reichersberg 42	2	4	9	2	9	2	2	2	2	2	8	*Yr7, Yr+*
	Haramoun	8	8	8	3	9	3	3	2	2	3	9	*Yr7*
	Tannour	1	8	2	1	2	2	2	2	2	2	8	*Yr7, Yr+*
	885	2	8	2	2	1	2	3	2	2	2	8	*Yr7, Yr+*
8	**Avocet *Yr*9/6*Avocet S**	2	8	2	2	2	8	8	8	8	8	8	*Yr*9
	Clement	1	5-6	2	1	2	8	8	8	8	8	9	*Yr*9
	Nab El Jamal	1	8	2	3	2	8	9	9	8	8	8	*Yr*9
10	**TP981**	2	8	9	9	9	8	8	9	9	9	9	
	Florence Aurore	1	8	8	8	9	9	9	9	8	8	8	*Yr*25
11	**Avocet *Yr*27/6*Avocet S**	5	8	3	2	3	3	2	3	2	2	3	*Yr*27
	Opata	5	8	3	2	3	3	2	3	2	2	3	*Yr*27
	MR 1009	3	8	2-3	2	2	3	2	2	2	2	5	*Yr*27
	Katilla	1	8	1	1	1	2	2	2	2	2	2	*Yr*27
	Bouhouth 6	3	8	3	2	3	2	2	2	2	2	4	*Yr*27
	Aammoun	1	8	1	1	1	2	3	2	2	2	5	*Yr*27
	Sham 8	2	8	2	1	2	2	2	3	2	2	5	*Yr*27
12	Lahn	8	7	1	5	2	1	2	6	3	4	8	*Yr*6+*Yr*7
	A1103	8	8	3	2	2	3	2	3	2	2	7	*Yr*6+*Yr*7
13	Senatore Cappelli	4	4	2	3	8	3	7	3	2	2	1	Ni[e]
	Miki	5	5	4	8	8	5	8	5	3	3	8	Ni
	Tal Amara 2	3	2	3	8	8	5	8	8	8	8	8	Ni
	Icarasha	4	4	2	8	2	2	2	2	4	2	8	Ni
	Tal Amara 1	4	3	1	8	2	2	1	4	2	2	2-4	Ni
	Tal Amara 3	6	5	2	6	2	2	2	4	4	4	8	Ni

[a]A=6E16, B=6E16V27, C=43E138, D=45E140, E=106E139, F=169E136V17, G=232E137, H=237E141, I=237E141V17, J=237E173V17, K=239E175V17. Pathotypes were coded according to Johnson *et al.* (1972). The virulences and avirulences tested were V1,2,3,4,6,7,8,9,17,25, 27,32,SD,SP,Su.

Scoring is done according to McNeal *et al.* (1971); Infection types IT0= No visible uredia, IT1= Necrotic flecks, IT2= Necrotic areas without sporulation, IT3-4= Necrotic and chlorotic areas with restricted sporulation, IT5-6= Moderate sporulation with necrosis and chlorosis, IT7-8= Sporulation with chlorosis, IT9= Abundant sporulation without chlorosis.

[b] Resistant to all *Puccinia striiformis* f. sp. *tritic*i pathotypes used

[c] - Corresponds to missing data

[d] Susceptible to all *Puccinia striiformis* f. sp. *tritici* pathotypes used

[e] Ni corresponds to non-identified resistance genes

[f] The entries in bold corresponds to the infection type profiles of the tester lines when confronted with the array of 11 *Puccinia striiformis* f. sp. *tritici* pathotypes

Forty-seven of the ICARDA elite lines evaluated for their resistance as seedlings were also tested as adult plants in a field test carried out in two locations, one in Syria (Tal Hadya) and

one in Lebanon (Terbol) (Table 5). There was imperfect concordance between the susceptible or resistant status of seedlings and adults. Some lines scored as susceptible in the seedling stage for the dominant inoculated pathotype prevalent in Lebanon and Syria showing virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27* and *YrSD* (Nazari et al., unpublished data), namely the elite lines 10, 11, 32, 35, 49, 71, 72 and 86, were resistant as adults. The adult elite line 64 was resistant to moderately resistant (30MR and 20R) in Tal Hadya and Terbol, respectively. The elite lines 65 and 66, which were resistant at the seedling stage, were only moderately resistant in the Lebanese field. The elite line 67 was resistant to all *Pst* pathotypes at the seedling stage and was also fully resistant in Tal Hadya but only moderately resistant to moderately susceptible in Terbol at the adult plant stage. The susceptible elite line 71 at the seedling stage was moderately resistant in the Syrian field. The elite line 72 susceptible at the seedling stage showed resistance in the Lebanese field. The elite lines 1 to 3 postulated to have *Yr1* showed similar intermediate resistance in both locations, rated 10-30MR and 20MR-MS in Tal Hadya and Terbol, respectively. The elite line 2 having also *Yr1* was moderately resistant to susceptible in the Lebanese field. The elite line 6 having *Yr*3 was resistant in the Lebanese field whereas the line 7 having *Yr4* was moderately resistant to susceptible. The elite lines 10 to 16 having *Yr6* were resistant in the fields considering that both locations had virulence to *Yr6*.suggesting carrying APR genes. The elite lines 19 to 26 having *Yr7* were resistant in the Lebanese field indicating APR in the lines at both locations since *Yr7* Avocet S had been always fully susceptible. The elite lines 34 and 39 having *Yr*17 were moderately resistant to moderately susceptible in the two locations. The two elite lines (41 and 42) postulated to have *Yr27* showed high severity in both locations and the line 43 was moderately resistant to susceptible in Lebanese field. Furthermore, the combination *Yr6*+*Yr17* provided resistance in Lebanese field for the lines 51 and 52 indication effectiveness of *Yr17* at both locations since the virulence for *Yr7* was common among the two races used for inoculations. The entry 55 postulated to have *Yr7*+*Yr4* showed high resistance in both locations indicating efficiency of the combination of the two seedling resistance genes. On the opposite, three elite lines (59-61) combining *Yr9*+*Yr4* were susceptible in the Lebanese field indicating the presence of *Vr9* and *Vr4* in the region; However the virulence to *Yr4* was not detected in the Lebanese field. The combinations *Yr9*+*Yr1*, *Yr9*+*Yr3*, *Yr7*+*Yr4* and *Yr6*+*Yr17* were resistant at adult plant stage in the Syrian

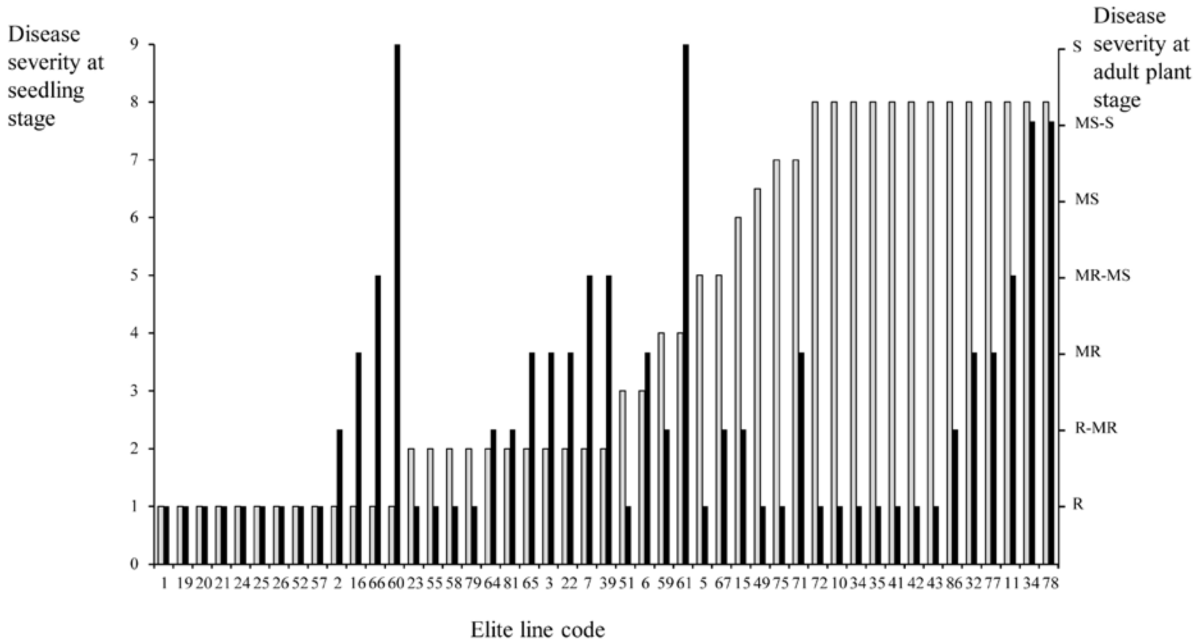

Figure 2. Disease severity at seedling reaction (Grey) and the adult plant severity (Black) of 47 bread wheat elite lines, confronted with the most dominant pathotype for the season 2010-2011 in Lebanon and Syria, carrying the *Vr2, 6, 7, 8, 9, 25, 27*, and *SD* based on pathotype surveys conducted by ICARDA for the same year. The disease severity at the seedling stage is scored from 0 to 9; 0 considered as resistant and 9 as susceptible (McNeal et al. 1971). At the adult plant stage, the disease severity was recorded according to Roelfs et al. (1992); R, MR, MS and S correspond to resistant, moderately resistant, moderately susceptible, and susceptible respectively. The code of elite lines is given in Table 5.

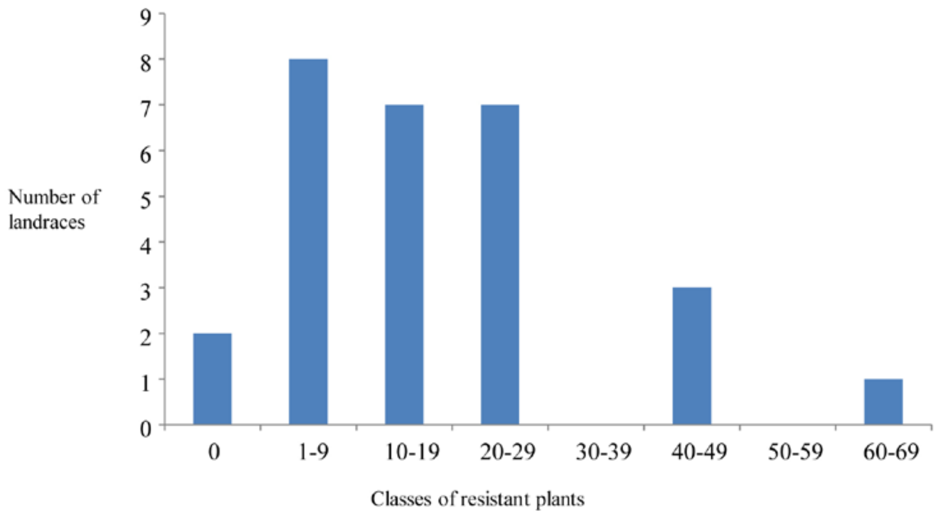

Figure 3. Distribution of resistance classes (Infection type up to 6) in the Lebanese landraces tested at the seedling stage with 11 races of *Puccinia striiformis* f. sp. *tritici*

field. Six lines having unidentified resistance genes, except the line 78, showed adult plant resistance in both locations. Since *Yr6*, *Yr7* and *Yr9* are largely deployed and virulences to these genes are confirmed, the combination of these genes with *Yr1*, *Yr3* and *Yr4* would be effective against the prevalent *Pst* pathotypes in the region (Fig. 2).

Resistance diversity in Lebanese landraces

Many landraces showed heterogeneous responses to the 11 *Pst* pathotypes, segregating for both resistant and susceptible plants. The segregation of landraces varied and showed diverse resistant classes towards the 11 *Pst* French races (Fig. 3). The highest number of resistant plants was observed with the pathotypes 6E16, 43E138 and 237E173 and the lowest proportion of resistant plants was observed with 239E175 combining the largest number of virulence factors. Interestingly, two durum wheat Bekaii (landraces 13 and 18) from two different locations and one bread wheat Naama (landrace 28) had a proportion of 100%, 11%, 75% of resistant plants, respectively when tested with the pathotype 239E175 (K) (Table 6). This indicates that these landraces carry new resistance gene(s) that could not be detected using set of pathotypes array. It was not possible to postulate which resistance gene was present in the resistant plants mixed in a susceptible landrace, but it indicated that unknown resistance was present and contributed to the resistance of the landraces, particularly when tested against the high virulence race 239E175.

Discussion

The host resistance approach remains the most economical and environmentally friendly method of controlling wheat rust diseases. Most of the characterised resistance genes are race specific, fitting the well-described gene-for-gene model (Flor 1956). Knowledge of the genetic structure of germplasm collections is crucial for efficient use of genetic resources namely in breeding programs. To identify the *Yr* genes in 138 ICARDA elite lines, Lebanese varieties and landraces, an array of 11 *Pst* pathotypes was used in the present study at the seedling stage. This set of pathotypes with complementary virulence spectra allowed us to infer the resistance profiles of most of the tested lines. The 11 *Pst* pathotypes in this study are able to discriminate 10 seedling resistance genes of which nine genes were postulated singly or in combination in tested genotypes. This indicates the usefulness of the pathotype array in detection of *Yr* gene in this study. However, we identified a group of genotypes for which seedling resistance could not be explained with the current *Pst* pathotypes and therefore their resistance characterization remained unclear. Among this resistance group, 9% of ICARDA

Table 4. Resistance group, infection types and postulated yellow rust resistance genes at the seedling stage towards 11 *Puccinia striiformis* f. sp. *tritici* pathotypes for 28 Lebanese landraces

Resistance group	Wheat line	Pathotype code[a]											Postulated
	Landrace	A	B	C	D	E	F	G	H	I	J	K	*Yr* genes
	Abou Shwereb	8	8	8	7	9	9	9	9	8	8	8	Susceptible[b]
	Abou Shwereb	8	8	8	8	9	9	9	9	8	8	9	Susceptible
	Salamouni	8	8	8	8	9	9	9	9	8	8	9	Susceptible
	Salamouni	8	8	8	9	9	8	9	8	8	8	9	Susceptible
	Salamouni	8	8	8	8	9	8	9	8	8	8	8	Susceptible
	Salamouni	8	8	8	8	9	8	9	9	8	8	8	Susceptible
	Salamouni	9	8	8	8	9	8	9	9	8	8	8	Susceptible
	Ukranian	8	8	8	8	9	8	9	9	8	8	9	Susceptible
	Ukranian	8	8	8	8	9	8	9	7	8	8	8	Susceptible
	Salamouni	9	8	8	9	9	9	9	9	7	8	9	Susceptible
2	Salamouni	8	8	8	9	9	8	9	9	8	8	8	Susceptible
	Abou Shwereb	9	8	8	9	9	9	9	9	8	8	9	Susceptible
	Salamouni	9	8	8	9	9	9	9	9	8	8	9	Susceptible
	Haurani	8	6-7	8	9	9	9	9	9	8	8	9	Susceptible
	Bekaii	7	8	6-7	7	9	8	9	8	8	8	9	Susceptible
	Salamouni	9	8	8	9	9	8	9	9	8	8	8	Susceptible
	Salamouni	8	8	8	9	9	8	9	9	8	8	8	Susceptible
	Salamouni	8	8	8	8	9	9	9	9	8	8	9	Susceptible
	Salamouni	8	8	8	8	9	9	9	9	8	8	9	Susceptible
	Salamouni	9	8	8	8	9	9	9	9	8	8	9	Susceptible
	Salamouni	8	8	8	8	9	9	9	9	8	8	9	Susceptible
	Vilmorin[c]	2	3	8	8	9	8	8	9	9	9	9	
3	Awnless variety	1	3	8	8	9	9	8	9	7	7	8	*Yr*3
	Abou Shwereb	3	4	8	8	9	9	9	9	8	8	8	*Yr*3
	Hybrid 46	2	1	1	2	9	2	8	9	9	9	8	
5	Nessr	2	4	2	5	9	5	9	9	8	8	8	*Yr*4
	TP981	2	8	9	9	9	8	8	9	9	9	9	
11	Salamouni	1	8	8	8	9	9	9	9	8	8	9	*Yr*25
	Salamouni	1	8	8	8	9	9	9	9	8	8	9	*Yr*25
12	Naama	1	4	2	2	2	2	2	5	8	8	8	*Yr*6 + *Yr*17
3	Bekaii	8	8	4	8	9	9	9	8	4	3	6	Ni[d]

[a]A=6E16, B=6E16V27, C=43E138, D=45E140, E=106E139, F=169E136V17, G=232E137, H=237E141, I=237E141V17, J=237E173V17, K=239E175V17. Pathotypes were coded according to Johnson *et al.* (1972). The virulences and avirulences tested were V1,2,3,4,6,7,8,9,17,25, 27,32,SD,SP,Su.

Scoring is done according to McNeal *et al.* (1971); Infection types IT0= No visible uredia, IT1= Necrotic flecks, IT2= Necrotic areas without sporulation, IT3-4= Necrotic and chlorotic areas with restricted sporulation, IT5-6= Moderate sporulation with necrosis and chlorosis, IT7-8= Sporulation with chlorosis, IT9= Abundant sporulation without chlorosis.

[b] Susceptible to all *Puccinia striiformis* f.sp. *tritici* pathotypes used

[c]The entries in bold corresponds to the infection type profiles of the tester lines when confronted with the array of 11 *Puccinia striiformis* f. sp. *tritici* pathotypes

[d] Ni corresponds to non-identified resistance genes

elite lines and 13% of Lebanese varieties showed complete resistance to all *Pst* pathotypes. Within the Lebanese resistant varieties, only three durum wheat genotypes were fully resistant whereas none of the Lebanese bread wheat varieties or bread or durum wheat landraces was completely resistant to all pathotypes.

In general, our study showed poor genetically based seedling resistance against relevant pathotypes from the CWANA area. Indeed, except for *Yr3* and *Yr4* and to some extent *Yr1*, which are effective in most of the wheat growing areas in CWANA, the rest of postulated genes confer no protection against current pathotypes. The *Yr1*, *Yr3*, and *Yr4* will no longer be effective when the North Western European pathotypes spread to CWANA region.*Yr1* was postulated only in 6% of ICARDA elite lines. Despite the effectiveness of *Yr1* in most of wheat growing areas in CWANA, virulence to the differential lines carrying *Yr1*, Chinese 166 (McIntosh et al. 2012) and Avocet/ 6* *Yr1*, was reported in East Asia (Stubbs, 1985), in Central Asia and the Caucasus region (Yahyaoui et al. 2002; Yahyaoui 2005) and Syria (K. Nazari unpublished data). Considering the specificity of *Yr1* and the spread of “Warrior” pathotype to North Africa and Turkey (www.wheatrust.org), the use of elite lines and commercial cultivars in CWANA with only *Yr1* has to be restricted because these will no longer be resistant to prevalent local pathotypes.

Yr3 and *Yr4* were infrequent in the tested lines although *Yr3* had been common in old North Western European varieties (de Vallavieille-Pope et al., 1990) and has been overcome in other regions such as Europe (Bayles and Priestley 1983; de Vallavieille-Pope et al. 2012), and Australia (Wellings 2011).

Yr6 was postulated singly or in combination with *Yr9* and *Yr17* in 13% of ICARDA elite lines and in 9% of Lebanese varieties in combination with *Yr7*. Varieties carrying *Yr6* were introduced into the CIMMYT wheat breeding program and hence to ICARDA germplasm as sources of leaf rust resistance, including *Lr13* and *Lr34* (Wellings 1986). However, this gene was not frequent in the ICARDA lines tested, and virulence to *Yr*6 is reported to be fixed for all tested isolates in Asia, Africa and South America (GRRC, 2014).

Yr7 was postulated singly only in one Lebanese variety (Haramoun) and in combination with additional resistance gene or genes in two other Lebanese varieties (Tannour and 885) and in 15 elite lines. *Yr7* originated from durum cv. Iumillo, was transferred to Thatcher wheat from which wheat cultivar Lee was derived (McIntosh et al. 2012). *Yr7* is present in a range of winter and spring wheat cultivars (McIntosh et al. 2012). This resistance gene has been

Table 5. Pedigree, seedling postulated stripe rust resistance genes and field responses of 47 bread wheat advanced lines from ICARDA to stripe rust at Tel Hadya and Terbol research stations in Syria and Lebanon, respectively.

Entry	Pedigree	Postulated *Yr* genes	Seedling[a] infection type	Adult plant resistance[b] Tal Hadya	Terbol
64	Tracha'S'//CMH76-252/PVN'S'	Resistant[c]	2	30MR	20R
65	Achtar*3//Kanz/KS85-8-4/3/Zemamra-5	Resistant	2	-[e]	10MR
66	Blass-1/4/CHAT'S'//KVZ/CGN/3/BAU'S'	Resistant	1	-	50R-MR
67	Crow'S'/Bow'S' -1994/95//Asfoor-5	Resistant	5	10R	30MR-MS
71	W3918A/JUP	Susceptible[d]	7	10-60MR	-
72	Nesma*2/14-2//2*Safi-3	Susceptible	8	-	5R
1	Crow'S'/Bow'S' -3-1994/95//Tevee'S'/Tadinia	*Yr*1	1	10-30MR	10R-MR
2	Tevee'S'/3/T.aestivum/SPRW'S'//CA8055/4/Pastor-2/5/Sunbri	*Yr*1	1	10-30MR	20MR-MS
3	Qafzah-2/Ferroug-2	*Yr*1	2	-	10MR
6	Bow #1/Fengkang15/3/HYS//DRC*2/7C	*Yr*3	3	-	10R
7	SHA3/Seri//Yang87-142/3/2*Towpe	*Yr*4	2	-	20MR-MS
10	Hamam-4/Angi-2	*Yr*6	8	-	10R
11	Hubara-16/2*Somama-3	*Yr*6	8	30R	-
5	MON'S'/ALD'S'//Towpe'S'	*Yr*6	5	-	10R
15	Weebill-1/2*Qafzah-21	*Yr*6	6	10R-MR	10R-MR
16	Rebwah-12/Zemamra-8	*Yr*6	1	1R	1R
19	Kauz//Kauz/Star	*Yr*7	1	15 MR	5R-MR
20	Cham-4/Shuha'S'/6/2*Saker/5/RBS/Anza/3/KVZ/HYS//YMH/TOB/4/Bow'S'	*Yr*7	1	-	5R
21	Cham-4/Shuha'S'/6/2*Saker/5/RBS/Anza/3/KVZ/HYS//YMH/TOB/4/Bow'S'	*Yr*7	1	-	5R
22	Florkwa-2/Asfoor-5	*Yr*7	2	-	5-30MR
23	Ferroug-2/Potam*2KS811261-8//Zemamra-8	*Yr*7	2	-	10R
24	Hubara-15/Zemamra-8	*Yr*7	1	-	5-10R
25	Cham-4/Shuha'S'/6/2*Saker/5/RBS/Anza/3/KVZ/HYS//YMH/TOB/4/Bow'S'	*Yr*7	1	-	5R
26	Shuha-5/Asfoor-1	*Yr*7	1	-	10R
32	MON'S'/ALD'S'//Aldan'S'/IAS58/3/Safi-1/4/Zemamra-1	*Yr*9	8	-	10MR
34	Clement/ALD'S'//Zarzour/5/AU//KAL/BB/3/BON/4/KVZ//CNO/PJ62 (Sandall 3)	*Yr*9	8	-	5R
35	ICARDA-SRRL-5	*Yr*9	8	-	20R
34	Clement/ALD'S'//Zarzor/5/AU//KAL/BB/3/BON/4/KVZ//CNO/PJ62 (Sandall 5)	*Yr*17	8	60MS-S	10R
39	Shuha-8//Vee'S'/Saker'S'	*Yr*17	2	-	40MR-MS
41	Kauz = JUP/BJY//URES	*Yr*27	8	100S	50S
42	Inqualab 91/Flag-2	*Yr*27	8	-	10S
43	Bow#1/Fengkang15/3/HYS//DRC*2/7C	*Yr*27	8	-	30MR-MS
49	GV/ALD'S'/5/ALD'S'/4/BB/G11//CNO67/7C/3/KVZ/TI/6/2*Towpe	*Yr*6+ *Yr*9	6-7	-	5R
51	DVERD-2/Aegilops squarrosa(214)//2*ESDA/3/NS732/HER	*Yr*6 + *Yr*17	3	-	5R
52	Hubara-3/Angi-2//Somama-3	*Yr*6 + *Yr*17	1	20R	-
55	Samar-8/Kauz'S'//Cham-4/Shuha'S'	*Yr*7 + *Yr*4	2	5R	10R
57	ACSAD 529/Karawan'S'//Somama-3	*Yr*9 + *Yr*1	1	5R	-
58	Hubara-5/3/SHA3/Seri//SHA4/Lira	*Yr*9 + *Yr*3	2	5R	-
59	Qafzah-33/Florkwa-2	*Yr*9 + *Yr*4	4	-	5R-10MR
60	Samar-12/Dollarbird	*Yr*9 + *Yr*4	1	-	50S
61	Milan/SHA7//Potam*3KS811261-5	*Yr*9 + *Yr*4	4	-	70S
75	T.aestivum/SPRW'S'//CA8055/3/Bacanora86	Ni[f]	7	1R	-
77	Fow-2/SD8036//Safil-3/3/NS732/HER//Kauz'S'	Ni	8	-	20MR
78	NS732/HER//Arrihane/3/PGO/Seri//BAU	Ni	8	-	40S-MS
79	IZAZ-2//Tevee'S'/Shuha'S'	Ni	2	-	20R
81	Sakha73/5/IAS 58/4/KAL/BB//CJ'S'/3/ALD'S'/6/Goumria-12	Ni	2	-	20R-MR
86	Girwill-13/2*Pastor-2	Ni	8	10R-MR	-

[a]Infection type towards 6E16V27, the most dominant pathotype in the CWANA region for the season 2010-2011, carrying the *Yr2, 6, 7, 8, 9, 25, 27*,and *SD* based on pathotype surveys conducted by ICARDA for the same year.
[b]R, MR, MS, S correspond to resistant, moderately resistant, moderately susceptible and susceptible, respectively according to the modified Cobb scale of Peterson *et al.* (1948). 5-100 corresponds to the percentage of leaf area covered by the disease. The scoring is the average of two and three seasons in the Syrian and Lebanese, respectively.
[c] Resistant to all *Puccinia striiformis* f. sp. *tritici* pathotypes used
[d] Susceptible to all *Puccinia striiformis* f. sp. *tritici* pathotypes used
e - Corresponds to missing data
[f]Ni corresponds to non-identified resistance genes

overcome in CWANA region and is no longer effective against the prevalent pathotypes identified in the region.*Yr9* was postulated singly in one Lebanese variety (Nab El Jamal) and in four ICARDA elite lines and in combination with *Yr1*, *Yr3* and *Yr4* in six elite lines. This gene which originated from *Secale cereale* is linked with *Lr26* and *Sr31* in the 1BL.1RS translocation (McIntosh et al. 2012). During the nineties, most of the wheat germplasm generated and distributed by CIMMYT throughout spring wheat production areas in low latitude countries carried the 1BL.1RS translocation (Bimb and Johnson, 1997). This translocation was identified in European wheat germplasm by Mettin et al. (1973) and Zeller (1973). The virulence against

Yr9 has been detected in wheat-growing areas since 1980s, especially in countries where CIMMYT nurseries were distributed starting from Ethiopia (Badebo and Bayu 1992), Syria (Mamluk and El-Naimi 1992), Turkey (Dusunceli et al. 1996), Iran (Torabi et al. 1995), Pakistan (Bahri et al 2011), and in Central Asia and Caucasus (Yahyaoui 2005) and the utilisation of this gene alone has to be restricted in the breeding materials.

Yr17 was postulated in four ICARDA elite lines and in combination with *Yr6* in Lebanese landrace (Naama). The cluster of genes *Yr17*, *Lr37* and *Sr38* was transferred to wheat in a translocation from *Aegilops ventricosa* (Dousssinault et al. 1998). Originally it was transferred to line VPM1 (a cross of *Aegilops ventricosa*, *Triticum persicum* and *cv*. Marne Desprez) (Bariana and McIntosh 1993). Virulence against *Yr17* has been detected in the USA (Line et al. 1992), in North Western Europe (Bayles et al. 2000; Hovmøller et al. 2002); The virulence to *Yr17* is currently frequent in the North Western European countries (de Vallavieille-Pope et al. 2012). The emergence of virulence to *Yr17* in Syria and Lebanon in recent years (Nazari K., personal communication) leads to the reduction of the utility of this *Yr* gene alone.

Yr25, which is common and overcome in North Western European varieties, was postulated in Lebanese landraces (2 populations of Salamouni), one Lebanese variety (Florence Aurore) and one elite line (40). Virulence against *Yr*25 is frequent in CWANA region (Yahyaoui et al. 2002).

Yr27 was postulated in four Lebanese varieties and eight ICARDA elite lines. This gene originated from the wheat cultivar Selkirk and was derived from *cv*. McMurachy (Wellings 1992), a parent of Selkirk. Again, this gene is present in many CIMMYT genotypes (Wellings 2011). Virulence for *Yr27* was found in New Zealand (Wellings and Burdon 1992), Pakistan (Bahri et al. 2011), India (Prashar et al. 2007), Tajikistan, Kyrgyzstan (Singh et al. 2004), Iran (Nazari and Torabi 2000) and Syria (Nazari et al. 2011).

Table 6. Frequency of resistant plants (R) among the susceptible landraces and percentage of susceptible plants (S) among the resistant landraces to each of the 11 French *Puccinia striiformis* f. sp. *tritici* pathotypes.

Landraces names	Locations	#	Pathotypes A % R	A % S	B % R	B % S	C % R	C % S	D % R	D % S	E % R	E % S	F % R	F % S	G % R	G % S	H % R	H % S	I % R	I % S	J % R	J % S	K % R	K % S
Abou Shwereb	Qamouaa, Akkar	1	26	-	-	26	34	-	19	-	17	-	19	-	23	-	9	-	30	-	50	50	0	-
Abou Shwereb	Qamouaa, Akkar	2	0	-	0	-	15	-	0	-	8	-	10	-	8	-	4	-	9	-	8	-	0	-
Salamouni	Qamouaa, Akkar	3	33	-	0	-	25	-	14	-	7	-	0	-	8	-	4	-	4	-	13	-	0	-
Awnless Variety	Qamouaa, Akkar	4	-	0	-	0	24	-	10	-	0	-	0	-	13	-	36	-	-	5	-	4	0	-
Abou Shwereb	Fneidik, Akkar	5	-	0	-	42	7	-	4	-	0	-	33	-	0	-	0	-	43	-	36	-	0	-
Salamouni	Fneidik, Akkar	6	-	48	17	-	33	-	8	-	26	-	13	-	26	-	0	-	29	-	30	-	0	-
Salamouni	Qamouaa, Akkar	7	50	-	28	-	23	-	-	46	0	-	0	-	0	-	0	-	-	19	26	-	0	-
Salamouni	Laklouk, Jbeil	8	8	-	9	-	19	-	-	41	0	-	29	-	0	-	17	-	-	42	-	36	0	-
Salamouni	Laklouk, Jbeil	9	50	50	32	-	13	-	7	-	9	-	34	-	0	-	0	-	35	-	50	50	0	-
Ukranian Variety	Tel Akhdar, Bekaa	10	-	46	0	-	11	-	3	-	0	-	9	-	0	-	50	50	0	-	0	-	0	-
Ukranian Variety	Tel Akhdar, Bekaa	11	37	-	13	-	16	-	0	-	12	-	18	-	0	-	38	-	4	-	0	-	0	-
Salamouni	Jeb Janine, Bekaa	12	13	-	0	-	-	28	0	-	0	-	0	-	0	-	0	-	32	-	33	-	0	-
Bekaii	Jeb Janine, Bekaa	13	12	-	22	-	-	31	23	-	4	-	37	-	12	-	50	50	-	0	-	20	-	0
Salamouni	Jeb Janine, Bekaa	14	0	-	11	-	22	-	0	-	4	-	16	-	0	-	11	-	-	46	20	-	0	0
Abou Shwereb	Aarida, Akkar	15	5	-	8	-	0	-	0	-	4	-	0	-	0	-	0	-	0	-	0	-	0	-
Salamouni	Aarida, Akkar	16	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Haurani	Tal Amara, Bekaa	17	0	-	-	4	17	-	0	-	0	-	0	-	0	-	0	-	0	-	22	-	0	-
Bekaii	Tal Amara, Bekaa	18	-	33	-	13	-	26	-	43	4	-	36	-	0	-	12	-	-	39	39	-	11	-
Salamouni	Northern Bekaa	19	0	-	0	-	-	0	0	-	4	-	5	-	0	-	0	-	0	-	0	-	0	-
Salamouni	Nabha, Bekaa	20	50	50	0	-	40	-	8	-	4	-	10	-	0	-	0	-	0	-	0	-	0	-
Salamouni	Nabha, Bekaa	21	-	0	0	-	4	-	4	-	4	-	0	-	0	-	4	-	4	-	4	-	0	-
Salamouni	Ham, Bekaa	22	7	-	0	-	7	-	0	-	0	-	7	-	9	-	0	-	4	-	4	-	0	-
Salamouni	Ham, Bekaa	23	7	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Salamouni	Aarsal, Bekaa	24	0	-	0	-	4	-	0	-	23	-	0	-	4	-	0	-	0	-	0	-	0	-
Salamouni	Northern Bekaa	25	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Salamouni	Northern Bekaa	26	-	0	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
Nessr	Tal Amara, Bekaa	27	-	0	-	0	-	-	0	-	8	-	0	-	0	-	0	-	0	-	0	-	0	-
Naama	Tal Amara, Bekaa	28	-	0	-	20	25	-	-	0	-	0	-	0	40	-	-	40	-	20	0	-	-	25

aA=6E16, B=6E16V27, C=43E138, D=45E140, E=106E139, F=169E136V17, G=232E137, H=237E141, I=237E141V17, J=237E173V17, K=239E175V17

Pathotypes were coded according to Johnson et al. (1972). The virulences and avirulences tested were *Vr1,2,3,4,6,7,8,9,17,25,27,32,SD,SP,Su*.

In Lebanon and Syria for the season 2010/2011, the genes *Yr1*, *Yr3* and *Yr4* were still effective. The virulences *Vr2*, *Vr6*, *Vr7*, *Vr*9, *Vr25*, and *Vr27* were predominant and *Vr8* and *Vr17* occurred only at low to intermediate frequencies (El Amil et al., in prep).

Adult plant resistance is often race non-specific and more durable than race-specific seedling resistance and was introduced in North American cultivars in 1950's (Chen 2007; Chen et al., 2014). A successful adult plant resistant cultivar *cv*-Gaines, selected in 1961 (Vogel 1964) had durable non-specific race stripe rust resistance. This approach prompted us to test adult plant resistance in Lebanese and Syrian fields for a subset of elite lines. The four lines having *Yr1* were moderately resistant to moderately susceptible in the field despite *Vr*1 not having been detected in the survey conducted in 2010-2011; therefore, *Vr*1 must have been present in Terbol (LB) at low frequency. The lines 15 and 16 having *Yr*6 were resistant and moderately resistant in the field despite the presence of *Vr*6 in Syria and Lebanon, indicating that this line must have additional adult plant resistance factors. The combination of the two seedling resistance genes *Yr*7+*Yr*4 was efficient in both locations giving that the combination *Vr4*+*Vr7* was absent in the region (El Amil et al., in prep). The line 19, with only *Yr*7, was less resistant in the field than the line 55 which has the combination *Yr*7+*Yr*4. The line 41 for which *Yr*27 was postulated singly was susceptible in Syria. The elite line 42 was susceptible in Lebanon (Missing data for Syria) whereas the line 43 was moderately resistant to moderately susceptible suggesting that line 43 showed adult plant resistance. The adult plant resistance test confirmed the presence of *Vr*27 in Syria and Lebanon in 2010 and 2012 as all lines harbouring *Yr27* showed susceptible reaction in the field.

Seedling resistance, based on a single resistance gene, alone is short lasting and rapidly overcome by the pathogen population. Combinations of multiple seedling resistances prolong the efficient life of a particular resistance gene but is rarely durable. Quantitative trait loci (QTLs) of adult plant resistance provide partial resistance but seldom confer early protection of the plant. Therefore, a combination of both types of resistance is crucial for the protection of the plant during its whole growing season. Cases of durable stripe rust resistance were observed in four French cultivars and one English cultivar combining both seedling resistance genes and QTLs at the adult plant stage: *cv*. Renan (Dedryver et al. 2009), *cv*. Camp Rémy (Mallard et al. 2005), *cv.* Apache (Paillard et al. 2012), *cv*. Soissons (de Vallavieille-Pope et al. 2012) and Claire (Powell et al. 2013). Among the tested lines having seedling and adult plant resistance, the lines 64, 65, and 66 are interesting cases to be explored.

Landraces are considered potential sources for disease resistance and agronomic traits. The large observed heterogeneity was already seen for plant height and days to heading in Israeli bread and durum wheat landrace populations (Beharav et al. 1997) and has been advocated as being potential sources of stripe and leaf rust resistance in nine Chinese landraces (Zhang 1995). This study indicated that each landrace was composed of several genotypes. Our study confirms that the landraces are composed of several genotypes and it will be particularly interesting to investigate resistance presence in the genotypes resistant to 239E175, the multi-virulent pathotype highly frequent in North Western Europe. Further study could indicate whether the resistance genes found in the landraces differed from the common genes already known and the resistant landraces could be exploited for rust resistance and other agronomic traits.

Acknowledgments

We highly thank Mr. Laurent Gérard and Mrs. Nathalie Galet for their technical assistance in sowing all the plant materials. We thank Jacqui Shykoff for her constructive comments which enabled improvements of the manuscript. We also thank Mr. Simon Khairallah for delivering the landrace seeds from his collection and preserved for the moment at the Gene Bank in LARI, Tal Amara. . This research was financed by ICARDA and the European Union Seventh Framework Programme (FP7/ 2007-2013) under the grant agreement n°265865-PURE.

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Appendix 1 : Names, pedigree, selection history and accession number of 87 elite lines of ICARDA, 23 Lebanese varieties and 28 Lebanese landraces

Entry Number	Name/type	Cross/pedigree and selection history
1	-	CROW'S'/BOW'S' -3-1994/95//TEVEE'S'/TADINIA ICW01-00260-0AP-6AP-0AP/0TS-0AP-8AP-1AP-0AP
2	-	**TEVEE'S'/3/T.AEST/SPRW'S'//CA8055/4/PASTOR-2/5/SUNBRI** ICW02-20046-4AP/0TS-0AP-0AP-11AP-0AP
3	-	**QAFZAH-2/FERROUG-2** ICW01-00205-0AP-6AP-0AP-0AP-3AP-0AP
4	Usher-16	**CROW'S'/BOW'S' -1994/95//ASFOOR-5** ICW01-00257-0AP-8AP-0AP/0TS-0AP-12AP-0AP
5	Settat-45	**FERROUG-2/POTAM*2KS811261-8//ZEMAMRA-8** ICW01-21116-2AP-3AP-0AP-0AP-9AP/MOR-0AP/MOR-0AP
6	-	**BOW #1/FENGKANG 15/3/HYS//DRC*2/7C** ICW02-00431-3AP/0TS-0AP-0AP-20AP-030KUL-0AP/0KUL-0DZ/0AP
7	Sanobar-1	**SHA3/SERI//YANG87-142/3/2*TOWPE** ICW00-0577-5AP-0AP-0AP-20AP-0AP
8	Sanobar-6	**SHA3/SERI//YANG87-142/3/2*TOWPE** ICW00-0577-7AP-0AP-0AP-5AP-0AP
9	-	**ESWYT99#18/ARRIHANE** MOROCCO F2-23 00/01-49AP-0AP-0AP-24AP/MOR-0AP/MOR-0AP
10	-	**HAMAM-4/ANGI-2** ICW02-00621-2AP/0TS-0AP-0AP-15AP-0AP
11	-	**HUBARA-16/2*SOMAMA-3** ICW03-20019-12AP-7AP/0TS-0AP-0AP-6AP-0AP
12	Zafir-3	**CHAM-4//SHUHA'S'/3/SD 8036** ICW98-0015-5AP-0APS-030AP-4AP-4AP-0AP
13	-	**AGUILAL/FLAG-3** ICW02-00295-22AP/0TS-0AP-030AP-3KUL-030KUL-0AP/0KUL-0DZ/0AP
14	Faisal-1	**MON'S'/ALD'S'//TOWPE'S'** ICW89-0237-1AP-0L-3AP-0AP-0Br-3AP-0AP
15	-	**WEEBILL-1/2*QAFZAH-21** ICW02-20182-12AP/0TS-0AP-0AP-2AP-0AP
16	-	**REBWAH-12/ZEMAMRA-8** ICW01-00193-0AP-4AP-0AP-0AP-6AP-0AP
17	Soonot-11	**SAMAR-8/KAUZ'S'//CHAM-4/SHUHA'S'** ICW01-21144-4AP-5AP-0AP-0AP-6AP-0AP
18	Neem-2	**SHUHA-4/6/GV/ALD'S'/5/ALD'S'/4/BB/GLL//CNO67/7C/3/KVZ/TI/7/PRL'S'/VEE'S'/3/P106.19//SOTY/** ICW01-21142-2AP-12AP-0AP-0AP-11AP-0AP
19	Cham-10	**KAUZ//KAUZ/STAR** CMBW90M4994-0T0PY-13M-015Y-015M-4Y-0B-0AP
20	Reyna-12	**CHAM-4/SHUHA'S'/6/2*SAKER/5/RBS/ANZA/3/KVZ/HYS//YMH/TOB** ICW00-0634-3AP-0AP-0AP-39AP-0AP
21	Reyna-25	**CHAM-4/SHUHA'S'/6/2*SAKER/5/RBS/ANZA/3/KVZ/HYS//YMH/TOB** ICW00-0634-6AP-0AP-0AP-48AP-0AP
22	-	**FLORKWA-2/ASFOOR-5** ICW01-00340-0AP-9AP-0AP/0TS-0AP-16AP-0AP
23	Settat-13	**FERROUG-2/POTAM*2KS811261-8//ZEMAMRA-8** ICW01-21116-1AP-8AP-0AP-0AP-10AP-0AP
24	-	**HUBARA-15/ZEMAMRA-8** ICW02-00017-5AP/0TS-0AP-0AP-20AP-0AP
25	Reyna-29	**CHAM-4/SHUHA'S'/6/2*SAKER/5/RBS/ANZA/3/KVZ/HYS//YMH/TOB** ICW00-0634-6AP-0AP-0AP-8AP-0AP
26	Sisaban-3	**SHUHA-5/ASFOOR-1** ICW00-0207-0AP-0AP-0AP-54AP-0AP
27	-	**ACHTAR*3//KANZ/KS85-8-4/3/LAKTA-8/4/ZEMAMRA-1**

Zhang ZJ (1995) Evidence of durable resistance in nine Chinese landraces and one Italian cultivar of *Triticum aestivum* to *Puccinia striiformis*. Eur J Plant Pathol 101:405–409

		ICW02-20073-12AP-0AP-0AP-0AP/MOR-0AP/MOR-0AP
28	Reyna-24	**CHAM-4/SHUHA'S'/6/2*SAKER/5/RBS/ANZA/3/KVZ/HYS//YMH/TOB** ICW00-0634-6AP-0AP-0AP-47AP-0AP
29	Laloub-2	**TURACO/CHIL/6/SERI 82/5/ALD'S'/4/BB/GLL//CNO67/7C/3/KVZ/TI** ICW99-0052-2AP-0AP-0AP-16AP-0AP
30	-	**ACHTAR/INRA 1764** MOROCCO F2-17 00/01-19AP-0AP-0AP-40AP/MOR-0AP/MOR-0AP
31	-	**ACHTAR/INRA 1764** MOROCCO F2-17 00/01-19AP-0AP-0AP-52AP/MOR-0AP/MOR-0AP
32	Haala-50	**MON'S'/ALD'S'//ALDAN'S'/IAS58/3/SAFI-1/4/ZEMAMRA-1** ICW01-21120-6AP-9AP-0AP-0AP-9AP-0AP
33	Battell-3	**SHUHA'S'/TAMEGA//BOW#1/FENGKANG15** ICW99-0180-2AP-0AP-0AP-2AP-0AP
34	Sandall-3	**CLEMENT/ALD'S'//ZARZOUR/5/AU//KAL/BB/3/BON/4/KVZ//CNO/PJ62** ICW99-0181-2AP-0AP-0AP-21AP-0AP
35	-	**ICARDA-SRRL-5** ICW88-0061-2AP-0L-5AP-0L-1AP-0TS-0AP
36	Ruth-1	**F5 DERIVED Kenya (D.H) F2** (F2 Kenya D.H #100)-0APS-030AP-1AP-1AP-0AP
37	Sandall-5	**CLEMENT/ALD'S'//ZARZOUR/5/AU//KAL/BB/3/BON/4/KVZ//CNO/PJ62** ICW99-0181-2AP-0AP-0AP-7AP-0AP
38	Nouha-3	**NS732/HER//MILAN/SHA7** ICW99-0288-15AP-0AP-0AP-25AP-0AP
39	Samira-2	**SHUHA-8//VEE'S'/SAKER'S'** ICW99-0387-23AP-0AP-0AP-35AP-0AP
40	Nayzak-3	**TEVEE'S'//KAUZ'S'/3/FOW'S'//NS732/HER** ICW98-0026-5AP-0APS-030AP-15AP-4AP-0AP
41	Cham-8	**KAUZ = JUP/BJY//URES** CM67458
42	-	**INQALAB 91/FLAG-2** ICW02-00354-9AP/0TS-0AP-0AP-17AP-030KUL-0AP/0KUL-0DZ/0AP
43	-	**BOW #1/FENGKANG 15/3/HYS//DRC*2/7C** ICW02-00431-3AP/0TS-0AP-0AP-19AP-030KUL-0AP/0KUL-0DZ/0AP
44	-	**NS732/HER//SD8036/3/SAADA** ICW00-0903-2AP-0AP-0AP-35AP-0AP-0AP
45	Loulou-16	**CBME4SA#4/FOW-2** ICW98-0047-1AP-0APS-030AP-8AP-5AP-1AP-0AP
46	HIJLEEJ-1	**SAKER/5/RBS/ANZA/3/KVZ/HYS//YMH/TOB/4/BOW'S'/6/PEWIT 3/7/ATENA-1** ICW01-21114-3AP-3AP-0AP/0TS-0AP-20AP-0AP
47	-	**NS732/HER*2//SAADA** ICW00-0885-8AP-0AP-0AP-6AP/MOR-0AP/MOR-0AP
48	Taleh-1	**TEVEE'S'/KAUZ'S'//ATTILA-5/6/GV/ALD'S'/5/ALD'S'/4/BB/GLL//CNO67/7C/3/KVZ/TI** ICW00-0626-5AP-0AP-0AP-16AP-0AP
49	Sidraa-1	**GV/ALD'S'/5/ALD'S'/4/BB/G11//CNO67/7C/3/KVZ/TI/6/2*TOWPE** ICW00-0629-1AP-0AP-0AP-16AP-0AP
50	Latifa-2	**PSN'S'/BOW'S'//KAUZ'S'/3/SAFI-1** ICW01-00489-0AP-12AP-0AP-0AP-6AP-0AP
51	Koukab-2	**DVERD-2/AE.SQUARROSA(214)//2*ESDA/3/NS732/HER** ICW98-0241-7AP-0APS-030AP-7AP-1AP-0AP
52	-	**HUBARA-3/ANGI-2//SOMAMA-3** ICW03-20005-2AP-10AP/0TS-0AP-0AP-10AP-0AP
53	Firdous-29	**GIZA-164/YEBROUD-1//BOOMA-2** ICW01-21194-5AP-4AP-0AP/0TS-0AP-7AP-9AP-0AP
54	Saamid-2	**GHURAB-2//TURACO/CHIL** ICW98-0226-9AP-0APS-030AP-9AP-6AP-0AP
55	Soonot-10	**SAMAR-8/KAUZ'S'//CHAM-4/SHUHA'S'**

		ICW01-21144-4AP-5AP-0AP-0AP-5AP-0AP
56	Nadia-13	**VAN'S'/3/CNDR'S'/ANA//CNDR'S'/MUS'S'/4/TEVEE-5** ICW99-0384-5AP-0AP-0AP-7AP-0AP
57	-	**ACSAD 529/KARAWAN'S'//SOMAMA-3** ICW03-0062-6AP/0TS-0AP-0AP-8AP-0AP
58	-	**HUBARA-5/3/SHA3/SERI//SHA4/LIRA** ICW03-0041-10AP/0TS-0AP-0AP-2AP-0AP
59	-	**QAFZAH-33/FLORKWA-2** ICW02-00423-10AP/0TS-0AP-0AP-16AP-0AP
60	Jelmoud-1	**SAMAR-12/DOLLARBIRD** ICW97-0503-8AP-0APS-3AP-0APS-030AP-0AP
61	-	**MILAN/SHA7//POTAM*3KS811261-5** ICW00-0028-0AP-0AP-0AP-6AP-0AP
62	Fanoos-14	**ANDALIEB-5//TEVEE-1/SHUHA-6** ICW01-00521-0AP-5AP-0AP-0AP-2AP-0AP
63	Tabeldi-1	**BUSH/AMIGO T101 X SAKHA 69//SHUHA-5/3/BOCRO-3** ICW00-0797-3AP-0AP-0AP-15AP-0AP
64	Babaga-3	**TRACHA'S'//CMH76-252/PVN'S'** ICW93-0065-2AP-0L-2AP-0L-0AP
65	Sale-6	**ACHTAR*3//KANZ/KS85-8-4/3/ZEMAMRA-5** ICW01-00135-0AP-1AP-0AP-0AP-7AP-0AP
66	Hashab-2	**BLASS-1/4/CHAT'S'//KVZ/CGN/3/BAU'S'** ICW00-0202-0AP-0AP-0AP-5AP-0AP
67	Usher-18	**CROW'S'/BOW'S' -1994/95//ASFOOR-5** ICW01-00257-0AP-8AP-0AP/0TS-0AP-9AP-0AP
68	-	**SABA/FLAG-1** ICW02-00301-21AP/0TS-0AP-030AP-3KUL-030KUL-0AP/0KUL-0DZ/0AP
69	Naji-3	**CBRD/KAUZ//WEEBILL 1/3/ATENA-1** ICW01-21107-1AP-10AP-0AP/0TS-0AP-8AP-0AP
70	-	**SHUHA-8/DUCULA** ICW99-0386-22AP-0AP-0AP-26AP/MOR-0AP/MOR-0AP
71	Cham-6	**W3918A/JUP** CM39992-8M-7Y-0M-0AP
72	-	**NESMA*2/14-2//2*SAFI-3** ICW00-0818-1AP-0AP-0AP-28AP/MOR-0AP/MOR-0AP
73	-	**SHUHA-8/DUCULA** ICW99-0386-22AP-0AP-0AP-54AP/MOR-0AP/MOR-0AP
74	-	**UTIQUE 96/FLAG-1** ICW02-00330-4AP/0TS-0AP-030AP-1KUL-030KUL-0AP/0KUL-0DZ/0AP
75	Hamam-4	**T.AEST/SPRW'S'//CA8055/3/BACANORA86** ICW92-0477-1AP-1AP-4AP-1AP-0AP
76	Durra-8	**FOW'S'//NS732/HER/3/CHAM-6//GHURAB'S'** ICW98-0035-5AP-0APS-030AP-7AP-5AP-0AP
77	-	**FOW-2/SD8036//SAFI-3/3/NS732/HER//KAUZ'S'** ICW01-21117-2AP-2AP-0AP-0AP-11AP-0AP
78	Temerind-8	**NS732/HER//ARRIHANE/3/PGO/SERI//BAU** ICW00-0904-3AP-0AP-0AP-29AP-0AP-0AP
79	Gonglase-4	**IZAZ-2//TEVEE'S'/SHUHA'S'** ICW01-00014-0AP-6AP-0AP-0AP-2AP-0AP
80	Bushraa-3	**PFAU/MILAN//CHAM-4** ICW98-0248-1AP-0APS-030AP-17AP-4AP-0AP
81	Jasmin-5	**SAKHA 73/5/IAS 58/4/KAL/BB//CJ'S'/3/ALD'S'/6/GOUMRIA-12** ICW01-00381-0AP-7AP-0AP/0TS-0AP-9AP-0AP
82	Qadanfer-5	**STAR'S'/FLORKWA-2** ICW97-0752-7AP-0APS-5AP-0APS-030AP-16AP-0AP
83	-	**TEVEE'S'/3/T.AEST/SPRW'S'//CA8055/4/PASTOR-2/5/SUNBRI**

18	Katilla, BW	-
19	Bouhouth 6, BW	-
20	Aammoun, BW	-
21	Lahn, DW	-
22	Miki, DW	Stj3//Bcr/Lks4 (ICD94-0994-C-10AP-0AP-2AP-0AP-9AP-0TR)
23	A1103, BW	Belikh//Gediz/Bit (ACS-D-7284-22IZ-14IZ-8IZ-OIZ)

BW and DW correspond to bread and durum wheat, respectively
- corresponds to missing data

CHAPTER 2

High pathotype diversity in a clonal population of the pathogen *Puccinia striiformis* f. sp. *tritici* responsible of wheat yellow rust in Lebanon and Syria

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Stripe (yellow) rust is the most important disease of wheat in the Central West Asia and North Africa (CWANA) region. Severe epidemics in 2010 caused more than 30% yield losses in Syria, and similar losses had occurred in Turkey, Uzbekistan and Iraq. Extensive field surveys were conducted in major bread and durum wheat areas in Lebanon and Syria in 2011 using the Borlaug Global Rust Initiative surveillance protocols. 275 samples of *Puccinia striiformis* f. sp. *tritici* from Lebanon and Syria were genotyped with 20 microsatellite markers. Fifty MLGs were structured into two closely related clonal subpopulations, one dominant in Lebanon and the other one in Syria. The subpopulation predominant in Syria was the most genetically similar to the 2004-2005 Middle East and Mediterranean population and had the genetic signature of PstS2 the aggressive strain tolerant to high temperature, worldwide spread since 2000. The pathotypes of 54 isolates had combinations of the virulences for the widely deployed genes *Yr2*, *Yr6*, *Yr7*, *Yr9*, *Yr25* and *Yr27*. Virulence for *Yr3*, *Yr8*, *Yr17* and *YrSP* occurred at varying frequencies. Resistance genes *Yr1*, *Yr4*, *Yr5*, *Yr10*, *Yr15* and *Yr32* were effective against all isolates.

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Six out of the ten pathotypes detected representing 53% of the isolates resembled to the PstS2 virulence profile.

Key words: wheat yellow (stripe) rust, pathotype, SSR, clonal populations

Introduction

Wheat yellow rust, caused by the fungal pathogen *Puccinia striiformis* f. sp. *tritici* (*Pst*) is a serious economic disease of wheat worldwide (Stubbs, 1985; de Vallavieille-Pope *et al*., 2012). This biotrophic pathogen historically occurred mainly in temperate areas with cool, humid summers or in high-altitude warm areas with cool nights (Rapilly, 1979). More recently this disease spread to warmer regions considered as harsh for the pathogen development (Høvmoller *et al*., 2011) and became important in Australia, New Zealand, and South Africa (Wellings, 2011). Severe epidemics occurred in wheat growing areas causing major losses ranking yellow rust the most serious biotic threat to wheat production (Wellings, 2011). A number of resistance genes to this pathogen are known, and managing host resistance remains the most economical and environmentally friendly measure for controlling the disease (Pathan & Park 2007). Severe epidemics are the result of new virulence emergence that overcome deployed resistance genes (Chen, 2007) and yield loss ranges from 10-70% (Chen, 2005). The Central West Asia and North Africa (CWANA) region witnessed many recurrent epidemics; there have been five in the last forty years. In Lebanon, in 1994, 30% of the national grain production was lost to yellow rust following the emergence of *Vr*7 (Mamluk, 1995) and in Iran, major epidemics were reported in 1995 and 2003 with the emergence of pathotypes with *Vr7* together with *Vr9*, and *Vr27*, respectively (Afshari *et al*., 2004). The two most recent epidemics, in Syria and Lebanon, were due to the emergence of the virulences *Vr9* in 1992 and *Vr27* in 2010 (Hodson & Nazari 2010, Morgounov *et al*., 2012).

Lebanon and Syria are located in the Near East Fertile Crescent, well known for the richness and diversity of wheat and its wild relatives (Harlan & Zohary 1966), which is expected to exert a selection pressure for the emergence of new pathotypes. Knowledge on the prevailing pathotypes is crucial, as pathogens like *Pst* evolve their virulence spectra frequently, thereby compromising the durability of resistance (McDonald & Linde 2002). More complex virulence combinations

can emerge through mutation, recombination, or migration over long distance (Wellings, 2011). The dispersal of *Pst* via uredospores over very long distances imply an urgent need to monitor *Pst* in all wheat growing regions and especially in hot spots (Brown & Hovmøller, 2002). Surveillance, monitoring and new virulence identification are prerequisites for future race prediction and for effective breeding programs (McIntosh et *al.,* 1995). Virulence/avirulence information on prevalent pathotypes is very useful for making decisions about which resistance genes to deploy locally.

In Syria, yellow rust has been reported annually since 1987 (Mamluk & El Naimi, 1992) and detailed monitoring in Syria and Lebanon was routinely carried out every year by planting the trap nurseries distributed from the International Center for Agricultural Research in the dry areas (ICARDA). Yahyaoui *et al.* (2002) studied the evolution of physiological races of *Pst* in Syria and Lebanon between 1994-1999. They observed high diversity of the *Pst* population, with 25 and 11 distinct pathotypes in Syria and Lebanon, respectively, and the emergence of new pathotypes during this five-year period, including the profile *Vr2*, *6*, *7*, *8*, *9* consistent with invasive high temperature tolerant strain described as PstS2 by Hovmøller *et al.* (2011). Bahri *et al.* (2009) studied the Mediterranean *Pst* populations. The pathotyping of 214 samples collected from Western part (Algeria, Italy, France, Morocco, Portugal, Spain and Tunisia) and 54 samples from Eastern part (Cyprus, Israel, Iran, Lebanon and Turkey) revealed twelve pathotypes. Eight of these were specific to the Eastern part, suggesting that the Eastern Mediterranean, because of its high diversity, is the source of new emerging strains. The pathotype which harbors *Vr2*, *6*, *7*, *8*, *9*, *25*, *27* and is tolerant to high temperature was first detected in Israel and later in the South of France, Italy, Portugal, Spain, Morocco, and Tunisia (Hovmøller *et al*., 2008, de Vallavieille-Pope *et al*., 2012).

Despite intensive monitoring described above, a detailed population genetic study of *Pst* in Lebanon and Syria was lacking. Lebanon and Syria are well known for their endemic *Berberis vulgaris* and *Berberis libanotica*, which were recently identified as the alternate host of *Pst* (Jin *et al*., 2010), for the richness of wheat wild relatives, and their high *Pst* diversity, even in a limited sample (Bahri *et al*., 2009). Therefore, this study was designed i) to study *Pst* pathotype diversity currently present across Lebanon and Syria and compare this with past reports; ii) to investigate *Pst* population genetic structure and test for evidence of recombination across

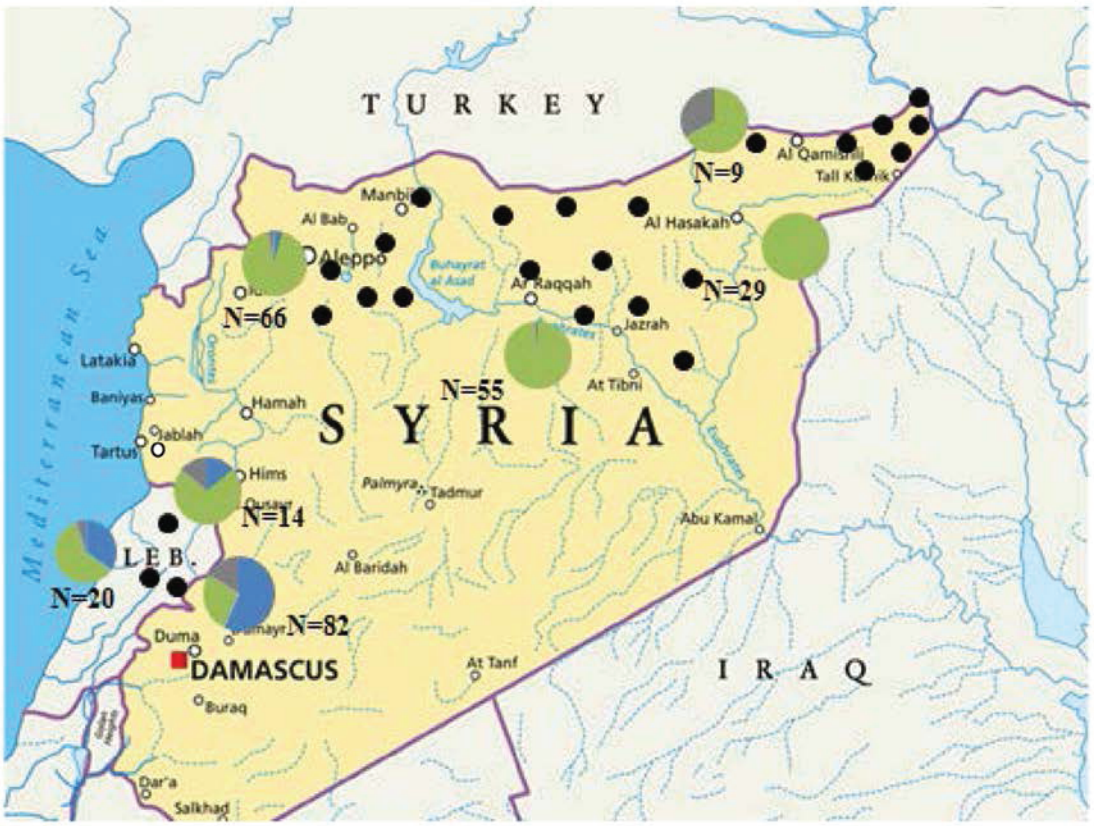

Figure 1: Map of the 22 Syrian and 3 Lebanese fields surveyed for *Puccinia striiformis* f. sp. *tritici* isolates along the main wheat production area in the Northern part of Syria in 2010-2011 (black dots). The frequency of subpopulation 1, subpopulation 2 and admixtures determined through analyzing 20 SSR marker data using STRUCTURE at K=4 are represented in blue, green, and grey, respectively.

Lebanon and Syria, given that the alternate host *Berberis* sp. is present in the region and iii) to test whether the aggressive strain PstS2 is still predominant in the region.

Materials and methods

Yellow rust sampling and spore multiplication

In May 2011, extensive surveys were conducted in the important wheat growing area in Syria. Sampling started from Aleppo where ICARDA is located, and was performed to the Eastern, Southern and Northern parts (Figure 1). In Lebanon, the survey was conducted in late May early June in the Bekaa valley, the center of wheat cultivation. Syrian samples were collected from 22 sites at different altitudes ranging from 209 to 501 meters above sea level (masl) and from major bread and durum wheat areas. Lebanese samples were collected from three sites at about 1000 masl from bread and durum wheat (Figure 1). At each site, 10-15 leaves with a single sporulating stripe lesion were collected, dried for 48 h at room temperature and kept in glassine bags at 4°C before spore multiplication.

Multiplication of the Syrian samples was carried out at ICARDA-Tal Hadya station (Syria) in Summer-Autumn 2011 and was successful for only 48 of the 159 samples. Multiplication of the Lebanese samples was carried out at INRA Versailles in May 2014 and was successful for only 6.

Single lesions were rubbed on 10-day-old wheat seedlings of the highly susceptible cultivar Morocco. The seedlings were placed for 24 h with 100% relative humidity at 10°C in a dew chamber in the dark to permit penetration of the fungus. Then the pots were placed in a climate chamber with a temperature regime of 19°C during 16 h of daylight at 300 µE m^{-2} s^{-1}, and of 16°C in the dark for 8 h. For mass spore multiplication, cv-Morocco was sown in trays (30 cm x 20 cm) and protected from airborne pathogens with plastic covers. When the seedlings were 1-cm tall, 60 ml of maleic hydrazide acid (0.25 g/l) was added. The inoculation took place under a laminar flow, which was decontaminated between each inoculation to avoid cross contamination. The incubation was done as described above. A week after inoculation, each tray was sealed within a cellophane bag to avoid cross-contamination. Eighteen days post-inoculation, uredospores were collected with a cyclone spore collector, dried in a desiccator filled with

Table 1: Twenty single *Yr*-genes wheat lines and the European and world set for differentiating virulence/avirulence of *Puccinia striiformis* f. sp. *tritici* pathotypes.

Yr gene	Single *Yr*-gene	> 1 *Yr* gene	[a]Genes
*Yr*1	AvS*Yr*1, NIL1, Chinese 161		
*Yr*2	Kalyansona	Heines VII, Heines Kolben	*Yr*2, *Yr*+
*Yr*3	Vilmorin 23	Nord Desprez	*Yr*3, *Yr*ND, *Yr*+
*Yr*4		Hybrid 46	*Yr*4, *Yr*+
*Yr*5	AvS*Yr*5, *Triticum spelta album*		
*Yr*6	AvS*Yr*6	Heines Kolben	*Yr*6, *Yr*2
		Heines Peko, Fielder	*Yr*6, *Yr*+
*Yr*7	AvS*Yr*7, Thatcher	Lee, Reichersberg 42	*Yr*7, *Yr*+
*Yr*8	AvS*Yr*8	Compair	*Yr*8, *Yr*19
*Yr*9	AvS*Yr*9, Fed.4/Kavkaz	Clement	*Yr*9, *Yr*Cle
*Yr*10	AvS*Yr*10	Moro	*Yr*10, *Yr*Mor
*Yr*15	AvS*Yr*15		
*Yr*17	AvS*Yr*17		
*Yr*25	TP1295, TP981		
*Yr*27	AvS*Yr*27, Ciano 79	Opata 85	*Yr*27, *Yr*18
		Attila CM85836-50Y	*Yr*27, *Yr*+
*Yr*32	AvS*Yr*32	Carstens V	*Yr*32, Yr+
*Yr*SD		Strubes Dickopf	*Yr*SD, *Yr*+
*Yr*Su		Suwon 92xOmar	*Yr*Su, *Yr*+
*Yr*SP	AvS*Yr*SP	Spaldings Prolific	*Yr*SP, *Yr*+
*Yr*ND		Nord Desprez	*Yr*3, *Yr*ND, *Yr*+
*Yr*A	AvSR, Inia 66	Anza	*Yr*A, *Yr*18

The tester genotypes comprised 15 world and European differential lines (Johnson et al., 1972), to which were added 11 wheat lines, and 13 Australian isogenic lines in the Avocet Near Isogenic lines background http://www.ars.usda.gov/SP2UserFiles/ad_hoc/36400500Resistancegenes/Yrgene.xls

[a]*Yr*+ corresponds to additional non-identified genes.

silicagel at 4ºC for 3 days, and stored in microtubes at -80ºC. From this multiplication, 15 mg of spores per isolate were conserved for further pathotyping and genotyping.

Virulence profile/pathotype determination

Pathotyping of the 48 successfully multiplied Syrian isolates took place at ICARDA station in Aleppo, Syria during Summer-Autumn 2011; the 6 multiplied Lebanese isolates were pathotyped at INRA Versailles in high confinement climatic rooms in May 2014. Their virulence profiles were determined using a robust set of 43 differentials, chosen from standard differential lines used worldwide. Most *Yr*-genes were represented in more than one tester genotype, and some tester genotypes harbored several resistance genes (Table 1). We assessed the reliability of our results by verifying that the compatibility of the interaction was coherent among the testers with the same resistance genes, singly or in combination. Cv-Morocco, Federation, Jupateco S, and Avocet S were used as susceptible controls. Ten seeds of each differential line were sown in square pots (7x7 cm). When ten-day old, the seedlings received a high-intensity light treatment prior to inoculation for at least 24 h to maximize infection success (de Vallavieille-Pope *et al*., 2002). Five mg of frozen uredospores received a heat shock of 35ºC for 5 mn, and then were suspended in 700 µL of mineral oil Soltrol 170 (Chevron-Phillips Chemical Co., Houston, USA) and sprayed onto ten two-leaf stage seedlings of each differential line. After 10 mn for oil evaporation at room temperature, the inoculated plants were incubated in a dew chamber at 10ºC for 24 h in the dark to ensure successful infection, and transferred to a climate chamber (day: 16 h, 300 µE m^{-2} s^{-1}, 19ºC; night: 8 h, 16ºC). Seedling infection types (IT) were recorded 15 to 17 days post-inoculation using a 0–4 scale where 0-2 indicate an incompatible reaction and 3 or 4 a compatible one as described by Wellings *et al.* (1988). An isolate was considered virulent if it produced 3 or 4 on all differential tester genotypes bearing a particular resistance gene. Here we gave an example of how we determined whether different isolates belonged to the same or to different pathotypes and therefore how we determined the pathotypes, considering the same *Yr* gene is carried by several differentials, singly or in combination. Here is the example for *Vr2* with the two differentials Kalyonsona and Heines VII. Of the 54 isolates, 47 showed strongly compatible reactions, six showed (Pathotype P8) only intermediate reactions and considered *Avr2*, and one interaction was missing on Kalyonsona, which carries only the *Yr*2 gene. On Heines VII, which also harbors *Yr*2 in addition to another anonymous resistance gene, only 17

isolates showed compatible reactions. Sixteen of those were also strongly compatible on Kalyansona, the last one being the missing value for Kalyansona. This demonstrates that the 17 compatible isolates on Heines VII carry *Vr2* and the anonymous virulence gene and the other 37 isolates that did not grow on Heines VII lack the virulence to the additional anonymous resistance gene. We applied this reasoning to every tested reaction in order to generate the set of pathotypes that explained our observations, taking into account the differential with the single *Yr* gene and considering the differential with additional gene as a confirmation.

DNA extraction and SSR genotyping

The molecular analyses were conducted at BIOGER-INRA, France. We genotyped all 275 Lebanese and Syrian samples using 20 microsatellite markers and following the protocols of Enjalbert *et al.* (2002) and Ali *et al.* (2011). In brief, DNA was extracted either from spores through the modified CTAB protocols (Enjalbert et *al.*, 2002) or from infected wheat lesions (Ali *et al.,* 2011) and was then amplified for the 20 microsatellite loci, previously developed, in three multiplex reactions using the Biorad thermocycler with 35 cycles of 95^{0}C for 30 s, 57^{0}C for 90 s and 72^{0}C for 30 s (Ali *et al*., 2011). Subsequently, the PCR products were separated using the Beckman Coulter CEQ-8000 DNA Analyzer. Electrophorograms were assessed using the CEQ-8000 Genetic Analysis System Software. Sixty three and 24 genotypes from the 2004-2005 Middle East and Mediterranean genetic group (Bahri *et al.,* 2009; Ali et *al.*, 2014a), and the North-Western European genetic group (Enjalbert *et al.*, 2005), respectively, were add as references to be compared with the Lebanese and Syrian samples collected in 2010-2011. Five MLGs from the 2004-2005 Middle East and Mediterranean collection corresponded to the MLG-99 reference that had the signature of the aggressive strain adapted to high temperature and spread worldwide (Ali *et al.*, 2014a).

Statistical analyses

The GENCLONE software (Arnaud-Haond & Belkhir, 2007) was used to assess the independent information given by each microsatellite locus and our ability to discriminate MLG (multilocus genotypes). The GENETIX 4.05.2 software (Belkhir *et al.*, 2004) was used to compute the number of alleles per loci, linkage disequilibrium among different loci by generating 1000 random permutations, and estimation of observed (Ho) and unbiased expected heterozygosity

Table 2: Virulence profiles and multilocus genotypes (MLG) of 48 Syrian and six Lebanese *Puccinia striiformis* f. sp. *tritici* isolates sampled in 2010-2011.

Pathotype code	*Yr*-gene[a]																	MLG[b]	Number of isolates on different host types[c]		
	Yr1	*Yr2*	*Yr3*	*Yr4*	*Yr5*	*Yr6*	*Yr7*	*Yr8*	*Yr9*	*Yr10*	*Yr15*	*Yr17*	*Yr25*	*Yr27*	*Yr32*	*YrSP*	*YrA*		BW	DW	Volunteer
Syria																					
P1	-	2	-	-	-	6	7	-	9	-	-	-	25	27	-	-	-	MLG-34 (2)	1	1	
P2	-	2	-	-	-	6	7	-	9	-	-	-	25	27	-	-	A	MLG-34 (16), MLG-11 (1)	12	4	1
P3	-	2	-	-	-	6	7	-	9	-	-	17	25	27	-	-	A	MLG-34 (3), MLG-18 (1), MLG-25* (1)		4	1
P4	-	2	-	-	-	6	7	8	9	-	-	-	25	27	-	-	A	MLG-34 (10), MLG-18 (2)	7	4	1
P5	-	2	-	-	-	6	7	8	9	-	-	-	25	27	-	SP	A	MLG-34 (2)	2		
P6	-	2	3	-	-	6	7	8	9	-	-	-	25	27	-	-	A	MLG-34 (1)			1
P7	-	2	-	-	-	6	7	8	9	-	-	17	25	27	-	-	A	MLG-34 (2), MLG-25* (1)	3		
P8	-	(2)	-	-	-	6	7	8	9	-	-	17	25	27	-	SP	A	MLG-34 (5), MLG-25* (1)	4	1	1
Lebanon																					
P9	-	2	-	-	-	6	7	8	-	-	-	-	-	-	-	-	-	MLG-nd[d] (1)	1		
P10	-	2	-	-	-	6	7	8	9	-	-	-	25	-	-	-	-	MLG-34 (1)	1		
P4	-	2	-	-	-	6	7	8	9	-	-	-	25	27	-	-	A	MLG-34 (4)	4		

[a]Virulences were determined using *Yr* genes in Table 1; - and gene number corresponded to the absence and presence of virulence, respectively. The gene number between brackets corresponded to intermediate infection type.

[b]MLG corresponded to multilocus genotypes grouped in the subpopulation 2 using 20 SSR markers under STRUCTURE at K=4 (Ali *et al.*, 2011). The number between brackets corresponded to the number of isolates.

[c]BW and DW corresponded to bread and durum wheat, respectively.

[d]nd missing data.

*corresponding to MLG differing by maximum 3 loci, RJN2, RJO21 and RJO27 from MLG-99, and carrying the signature of PstS2 aggressive strain on the SSR marker WU12 (325/334).

(He). Deviation from Hardy-Weinberg equilibrium using GENPOP 4 and genotypic diversity statistics (including numbers of specific alleles and specific MLGs, and Shannon index) using GENCLONE, as well as, Pairwise Nei genetic distances and pairwise FST statistics using GenALEx software (Peakall & Smouse, 2012) were estimated. Calculations were performed among Lebanese and Syrian populations, among the genetic subpopulations detected by STRUCTURE, and among hosts. The level of population differentiation was also performed among the 7 different geographically spaced populations.

Principal Coordinates Analysis (PCoA), using GenALEx software (Peakall & Smouse, 2012), was performed on SSR data from the 2010-2011 Lebanese and Syrian isolates, as well as references from the 2004-2005 Middle East and Mediterranean, and North Western European populations. We tested whether there was genetic substructure in the samples using the model-based Bayesian method implemented in the STRUCTURE 2.2 software (Pritchard *et al.,* 2000), which assigns MLGs to clusters while minimizing the Hardy–Weinberg disequilibrium and gametic-phase disequilibrium among loci within clusters. A total of 10 independent runs were made with a burn-in period of 100 000 and a total of 100 000 iterations, selecting K ranging from 1 to 10. STRUCTURE outputs were processed with CLUMPP (Jakobsson &Rosenberg 2007) to assign groups of runs to a common clustering pattern (G'-statistic greater than 80%). The optimal K value was determined using the method of Evanno *et al*. (2005) based on the rate of change in the log probability of data between successive K values. Calculations were done on clone corrected data, with one individual MLG.

Results

Virulences in Lebanon and Syria

We pathotyped 54 isolates, 48 Syrian and six Lebanese, using wheat lines differentiating 20 yellow rust *Yr* resistance genes, singly or in combination with one or more additional genes. We inferred 10 pathotypes for the whole sample, eight in Syria and three in Lebanon, with one common pathotype P4 in both countries (Table 2). The pathotype P2 with 17 isolates was dominant in Syria and shared the dominant virulence profile *Vr*2, *6*, *7*, *9*, *25*, *27*, *A*. The pathotype P4, common to Syria and Lebanon, was also frequent with 16 isolates in total, had *Vr*8 in addition to the virulences carried by P2. The most frequent pathotypes P2 and P4 were found

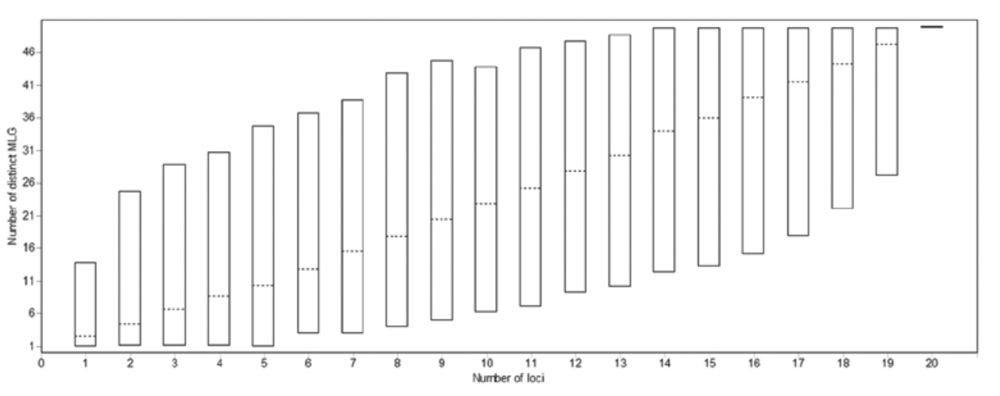

Figure 2: Number of *Puccinia striiformis* f. sp. *tritici* multilocus genotypes (MLG) detected in 2010-2011 population from Lebanon and Syria under GENCLONE software. The box represents the average, minimum and maximum numbers of MLGs detected when re-sampling on the 20 microsatellite loci using 1000 permutations.

Table 3: Number of alleles, expected (He) and observed heterozygosity (Ho), and the allele size range estimated for 20 SSR markers in the *Puccinia striiformis* f. sp. *tritici* population sampled in 2010- 2011 in Lebanon and Syria. Reference isolates from the 2004-2005 Middle East and Mediterranean population, and the 2008 North-Western European population.

SSR locus	No of alleles	Expected heterozygosity	Observed heterozygosity	Allele size range
RJN2	11	0.69	0.28	162-192
RJN3	3	0.21	0.01	339-343
RJN4	2	0.49	0.91	260-262
RJN5	3	0.53	0.96	228-232
RJN6	2	0.14	0.15	316-318
RJN8	2	0.49	0.89	306-309
RJN9	2	0.08	0.08	335-337
RJN10	3	0.54	0.92	225-231
RJN11	7	0.62	1.00	175-185
RJN12	1	0.00	0.00	198
RJN13	2	0.49	0.89	150-153
RJO3	2	0.51	0.96	202-204
RJO4	3	0.60	1.00	201-207
RJO18	3	0.54	1.00	334-360
RJO20	2	0.16	0.02	287-290
RJO21	4	0.57	0.92	173-183
RJO24	13	0.76	0.96	274-305
RJO27	4	0.31	0.24	229-243
WU6	2	0.03	0.01	212-213
WU12	2	0.45	0.65	325-334
Mean	3.65	0.41	0.59	

in 31% and 30% of the whole population, respectively. P9 and P10 were unique to Lebanese samples having *Vr2*, *6*, *7*, *8* and *Vr2*, *6*, *7*, *8*, *9*, *25*, respectively.

Eleven *Yr*-genes out of 20 tested were overcome by at least one isolate. The isolates harbored between 4 and 9 different virulences; the most complex virulence spectrum with nine virulences was P8 and represented 11% of the population. The most frequent pathotypes P2 and P4, as well as the most complex pathotype P8 were found on all types of hosts, on bread wheat, durum wheat, and volunteer (Table 2). All the isolates had in common the virulences *Vr6* and *Vr7*. The virulences *Vr2, Vr9*, *Vr25*, *Vr27* and *VrA* were highly frequent in the population with frequencies ranging from 92%, to 98%. The virulence *Vr17* was present in 14 isolates belonging to three pathotypes (P3, P7 and P8). *VrSP* was present in 8 isolates belonging to two pathotypes (P5 and P8). The *Vr3* appeared in only pathotype P6, represented by a single isolate. Interestingly, two Syrian isolates belonging to P1, having the most frequent virulences *Vr2*, *6*, *7*, *9*, *25*, *27*, one recovered from bread wheat and the other from durum wheat, showed low infection type on Avocet S which was susceptible for all other isolates. The virulences *Vr1*, *Vr4*, *Vr5*, *Vr10*, *Vr15* and *Vr32* were clearly absent, because we saw no evidence of a compatible reaction on any genotype tester bearing the relevant resistance genes. For genotype testers with *YrSD*, *YrSu* and *YrND*, there were some intermediate reactions. However we chose not to interpret intermediate reactions as sufficient evidence for the presence of virulence genes. Therefore we can claim to have not detected *VrSD*, *VrSu* and *VrND*.

Population genetic structure

We genotyped 275 *Pst* isolates, 116 Lebanese and 159 Syrian, using a set of 20 microsatellite markers. These markers varied in their information content, having between one to 13 alleles (Table 3). The number of multilocus genotypes (MLG) detected increased steadily when using information from more loci without showing a plateau (Figure 2). The 20 microsatellite markers allowed us to discriminate 50 MLGs, 30 in Lebanon and 26 in Syria (Table 4), that are identical for ten SSR loci and the other ten markers showed different level of polymorphism (RJN2, RJN3, RJN5, RJN6, RJN10, RJN13, RJO4, RJO24, RJO27 and WU12). We found strong linkage disequilibrium for several pairs of loci (Supplementary Table 1). Of the 50 MLGs, 35 were found only once: 22 in the Lebanese samples and 3 in the Syrian ones. The dominant MLG-34 represented 60% of the Syrian samples and 22% of Lebanese ones (Figure 3). The dominant

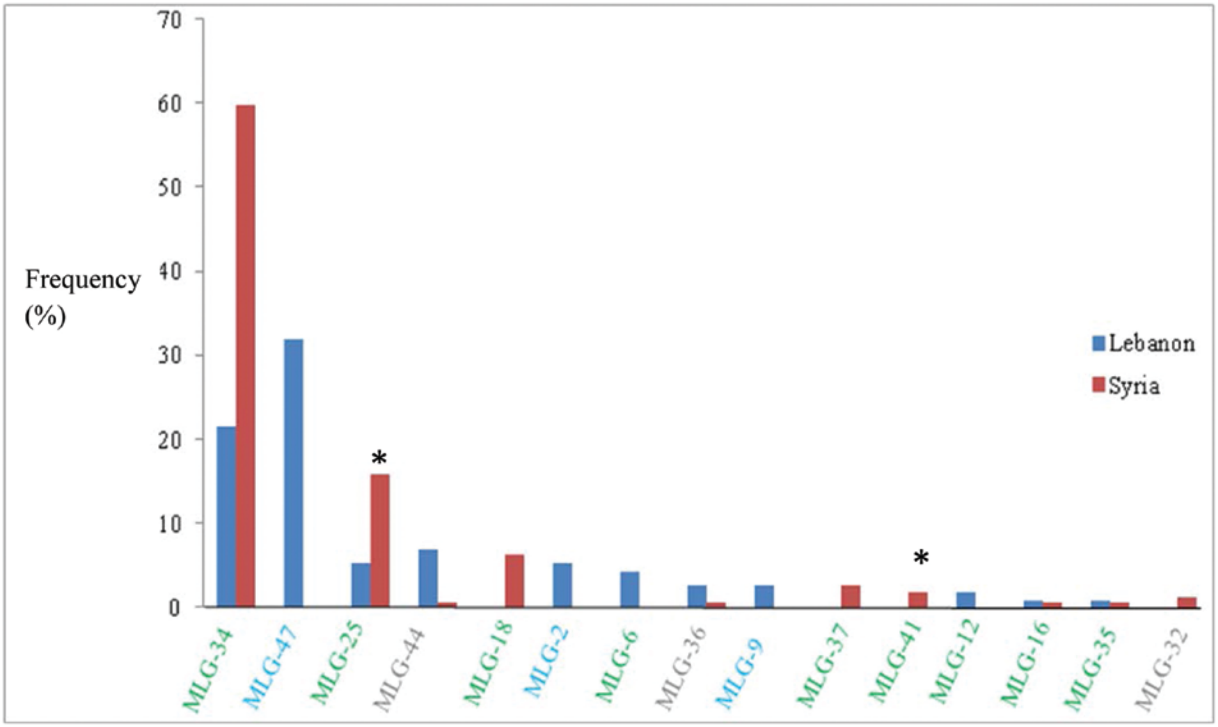

Figure 3: Frequency of the 15 most frequent multilocus (MLG) genotypes based on 20 microsatellite markers for *Puccinia striiformis* f. sp. *tritici* isolates sampled in 2010-2011 in Lebanon and Syria; 35 MLGs sampled once were not included. MLGs' names are color coded according to their genetic group determined using 20 SSR markers under STRUCTURE at K=4: MLG belonging to subpopulation 1, subpopulation 2 and admixtures are represented in blue, green, and grey, respectively. * corresponded to MLG differing by maximum 3 loci, RJN2, RJO21 and RJO27 from MLG-99, and carrying the signature of PstS2 aggressive strain on the SSR marker WU12 (325/334).

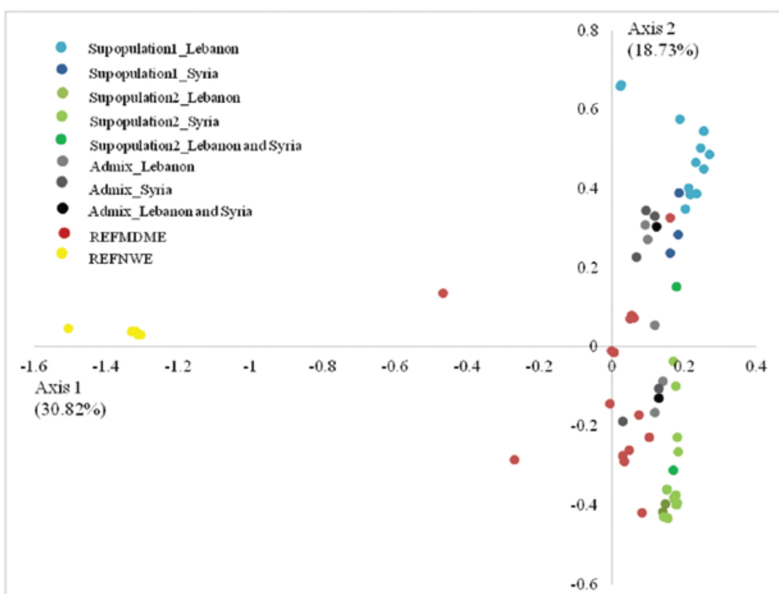

Figure 4: Principal Coordinates Analysis (PCoA) based on SSR data of *Puccinia striiformis* f. sp. *tritici* isolates sampled in Lebanon and Syria in 2010-2011, Middle East and Mediterranean area (REFMDME) in 2004-2005 (Bahri *et al.*, 2009), and North-Western European area (REFNWE) in 2008-2009 (Enjalbert *et al.*, 2005). The isolates were classified according to the origin country and subpopulation group defined by STRUCTURE using 20 SSR markers.

MLG-47 in Lebanon represented 32% of the Lebanese samples but was not detected in Syria. Only five resampled MLGs were found exclusively in the Lebanese population, four in the Syrian population, and six MLGs were common for the two populations corresponding to 168 isolates (Figure 3). Two MLGs, MLG-18 and MLG-47, representing 17% of the population, differed from the dominant MLG-34 by one and two alleles, respectively.

Comparing SSR profiles between our samples and 63 Middle East and Mediterranean samples from 2005-2006 (Bahri *et al.,* 2009; Ali *et al*., 2014), we found differentiation between the 2004-2005 and “2010-2011” population. None of the 50 MLGs detected in Lebanon and Syria in this study were found in the 63 isolates sampled in the Middle East and Mediterranean area in 2005-2006. Four of the 50 MLGs (MLG-24, MLG-25, MLG28, and MLG-41), representing 19.5% and 4% Syrian and Lebanese isolates respectively, differed from MLG-99 reference sampled in the Middle East and Mediterranean area in 2005-2006, by maximum 3 loci (at RJN2, RJO21 and RJO27) and carrying the signature of PstS2 aggressive strain at the SSR marker WU12 (325/334). However, of the 20 SSR markers, 6 SSRs were monomorphic between the old Middle East and Mediterranean population and the recent Lebanese and Syrian population.

Clustering analyses with STRUCTURE identified a clear pattern of population subdivision. We tested for a number of clusters ranging from 1 to 10 and found the best result for K =4 (Figure S1) (Evanno *et al*., 2005). At the optimal value for the number of clusters K=4, the 38 MLGs from Syria and Lebanon clustered into two subpopulations, subpopulation 1 and subpopulation 2, and 12 admixed individuals. In addition, the isolates from the 2004-2005 Middle East and Mediterranean, and North Western European genetic groups used as references individualized into clear separate clusters (Figure S2). The STRUCTURE analysis was able to distinguish the North Western European population at K=2 followed by the 2004-2005 Middle East and Mediterranean populations at K=3. Higher proportion of admixtures between subpopulation 1 and subpopulation 2 was observed in Lebanon than in Syria (Figure 1; Table 4). Principal Coordinates Analysis (PCoA) discriminated the subpopulation 1, the subpopulation 2, and North Western European isolates in distinct groups; the admixures were scattered between the two subpopulations from the 2010-2011 season (figure 4) The 2004-2005 Middle East and Mediterranean references were genetically closer to the subpopulation 2 than the subpopulation 1 as confirmed by Nei genetic distance and Fst (Supplementary Table 3).

Table 4: Gene and genotype diversity for Puccinia striiformis f. sp. tritici isolates collected in 2010-2011 based on genetic subpopulation, country, and host type.

	Population	Subpopulation	Subpopulation	Country		Host			
	2010-2011	1	2	Lebanon	Syria	Bread wheat	Durum wheat	Triticale	Volunteer
Sample size	275[a]	59 (56/3)[b]	193 (44/149)[b]	116	159	203	61	3	8
Gene diversity	0.340	0.304	0.309	0.343	0.305	0.342	0.344	0.300	0.300
No of alleles/locus	2.600	2.150	2.350	2.263	2.421	2.65	1.95	1.6	1.6
No of alleles	52	43	47	45	50	46	40	32	32
Allelic richness	1.615	1.595	1.634	1.737	1.633	1.740	1.740	1.600	1.60
No. of MLGs	50 (30/26)[c]	16 (13/3)[c]	22 (10/16)[c]	30	26	44	11	1	1
No of MLGs related to MLG-99[d]	4	0	4	1	4	4	1	0	0
Fst		0.102		0.031		0.005			
Nei's genetic distance		0.138		0.035		0.004			
Fis	-0.526	-0.434	-0.681	-0.538	-0.575	-0.507	-0.547	-1	-1
Ho	0.554*	0.550*	0.599*	0.568*	0.596*	0.589*	0.575*	0.6*	0.6*
He	0.343	0.304	0.309	0.337	0.315	0.342	0.343	0.300	0.300
Shannon index	2.346	1.574	1.476	1.632	2.441	2.650	1.950		

[a]23 isolates belonged to admixture group.

[b](X/Y): X corresponded to the number of isolates in Lebanon/ Y number of isolates in Syria.

[c](X/Y): X corresponded to the number of MLGs in Lebanon/ Y number of MLGs in Syria.

[d]MLG differing by maximum 3 loci, RJN2, RJO21 and RJO27 from MLG-99, and carrying the signature of PstS2 aggressive strain on the SSR marker WU12 (325/334).

* were significantly different from expected heterozygosity at P=0.05.

Subpopulation 1, representing 21.5% of the genotyped isolates, was mainly present in Lebanon at 95% and only 5% in Syria, while subpopulation 2 representing 70% of the genotyped isolates, was mainly present in Syria at 77% and only 23% in Lebanon (Table 4). Subpopulation 1 and subpopulation 2 showed an excess of observed heterozygosity and strong linkage disequilibrium for the 20 SSR markers and were therefore considered that *Pst* population in Syria and Lebanon as clonal (Table 4). The two subpopulations 1 and 2 were monomorphic with 10 SSR markers depicting their strong genetic proximity. The Nei genetic distance between subpopulation 1, subpopulation 2, and the 2004-2005 Middle East and Mediterranean population was estimated to be around 0.1 whereas the genetic distance of the North-Western European population was around 0.5 (Table 4). The Fst was ranging from 0.051 to 0.102 between subpopulation 1, subpopulation 2, and the 2004-2005 Middle East and Mediterranean population whereas when compared to the North-Western European, Fst was comprised between 0.292 to 0.293 (Supplementary Table 3). Similarly, low genetic differenciation was observed between different types of host: Bread wheat, durum wheat, Triticale, and volunteer (Table 4). Low genetic diversity was observed across the 7 locations and in the overall populations (Supplementary table 2). When comparing pathotypes and MLGs, MLG-34 was dominant in at least nine pathotypes at high frequency varying from 83% to 85% in the pathotyped Syrian, Lebanese, and 2010-2011 population (Table 2). MLG-18 was unique in Syria (4% of pathotyped isolates) and was common between P3 and P4.

Discussion

Surveillance has been traditionally used for depicting new virulent race emergence. Worldwide, pathotype variability stands behind the recurrent yellow rust epidemic by the outbreak of specific Yr genes. We identified ten pathotypes out of 54 Syrian and Lebanese isolates surveyed in 2010-2011. The previous studies reported 14, 16, and 8 pathotypes for the periods 1994-1995, 1998-1999, and 2004-2005 respectively (Yahyaoui *et al*., 2002; Bahri *et al*., 2009;) and similarly depicted a high pathotype diversity. The sampling differed between the studies: Bahri *et al*. (2009) received samples from collaborators from six countries, Yahyaoui *et al.* (2002) sampled mainly in trap nurseries and here we surveyed the whole wheat growing part of Syria and got limited sampling from Lebanon. Comparison of recent pathotypes with the previous ones showed that the number of virulences had increased over time. In CWANA region, culture

system included several types of wheat genotypes: landraces, farmers varieties and modern selected varieties for both bread and durum wheat. Furthermore, no fungicide sprays were applied in the region. Those selection pressures might contribute to the selection of diverse pathotypes in the pathogen population. Six pathotypes from both countries (representing 41% of the pathotyped isolates), P4, P5, P6, P7, P8, and P10 shared similarity in their virulence profile with the aggressive and tolerant to warm temperature strain PstS1/PstS2, and often combined with *Vr*25 and/or *Vr*27 (Hovmøller *et al*., 2011). PstS2-like pathotypes were frequent in the year 2005-2006 for the Middle East and Mediterranean, and also dominated the year 2010-2011. The two aggressive strains PstS1/PstS2 were present in the Middle East, in Iran (Afshari *et al*., 2004) and in North Africa (Bahri *et al*., 2009). Except for *Vr17*, all depicted virulences in this study were already reported previously in Israel, in South of France, Italy, Portugal, Spain, Morocco, and Tunisia (Bahri *et al*., 2009). The fixed virulences *Vr6* and *Vr7* from 1999, were the most frequent followed by *Vr2*, *Vr9* and *Vr27*. In Lebanon and Syria, the season 2010-2011 witnessed the emergence of *Vr27* resulting in severe epidemics and yield losses for both countries. The resistance genes *Yr6* + undetermined gene, *Yr7* +, and *Yr27* were the most frequent postulated resistance genes on a subset of varieties from the International Center for Agricultural Research in Dry Areas (ICARDA) and in Lebanese varieties (El Amil *et al*., in preparation). *Vr8* frequency was only 55% in the 2010-2011 Lebanese and Syrian population and was not fixed in Syria although this virulence was described in PstS1/S2 strains worldwide. *Vr17* was reported for the Syrian population at only 26%, and absent in the Lebanese population, probably due to the limited number of pathotyped isolates. *Vr17* virulence was not detected by Bahri *et al*. (2009) in the Mediterranean region; Yahyaoui *et al*. (2002) did not include the differential host genotype carrying *Yr17* (AvSYr17 or VPM1). These outcomes also agreed with gene postulation results. *Yr8* was not postulated and *Yr17* was present at very low frequency in the CWANA lines tested (El Amil *et al*., in preparation).

Based on avirulence of Avocet S towards two Syrian isolates, we postulated that Avocet S carries at least one effective gene for resistance to the two isolates (temporarily designated as *YrAvS*). This suggested that Avocet S was not genetically homogeneous or more likely, that the genetic background of Avocet S was not fully regenerated during the six backcross and subsequent selfing generations. The resistance of Avocet S is yet to be characterized (Nazari & El Amil, 2013). These results illustrated the problems that can arise when sets of differential

genotypes that perform in a satisfactory way in a particular limited geographical area are applied globally. *Pst* races carrying avirulence genes that are absent in the original area where a differential cultivar was characterized for its susceptibility may be present in other areas. Consequently, a differential cultivar which was susceptible in the original area may unexpectedly exhibit resistance in a new region. This was also reported for Victo, which has been reported universally susceptible to North-Western European *Pst* population, showed avirulence to Pakistani isolates (Ali *et al*., 2014b). This was also reported for wheat lines more resistant to an exotic *Pst* strain than to the local ones (Sørensen *et al*., 2014). Therefore, host lines considered to be "universally susceptible" in one region may not be susceptible globally.

Genotyping 275 Syrian and Lebanese *Pst* isolates revealed an overall clonal population structure with some admixed individuals. Differentiation was not observed between the two countries. The genotyping analyses showed a genetic proximity of the 2010-2011 Syrian and Lebanese *Pst* samples with the 2004-2005 Middle East and East Africa genetic group described by Bahri *et al*., (2009) and Ali *et al*. (2014a). The 2010-2011 population clustered between subpopulation 1 dominant in Lebanon and subpopulation 2 dominant in Syria. This clear subdivision between Lebanon and Syria might be due to geographical barrier of high mountainous chain between the two countries or the host structure specific to each country. Although Lebanon presented both subpopulations at almost similar amount, while Syria was mainly represented by subpopulation2, both countries were genetically close. The presence of the alternate host of yellow rust did not seem to contribute in having more diverse meta-population in Lebanon since the wheat growing area in Lebanon was centralized in the Bekaa valley surrounded by mountains having the Berberis sp. plantations at high altitude above 1200 masl. The role of Berberis sp. for *Pst* life cycle under natural conditions which required synchrology between the liberation of *Pst* basidiospores and occurrence of young leaves of Berberis remains to be further investigated (Wang & Chen, 2015). The Northern part of growing area in Syria was mostly delimited from the South by the desert. Moreover, Lebanon was growing more durum wheat than bread wheat opposing to Syria in which bread wheat was mainly cultivated. In Lebanon, farmers continue to cultivate old wheat landraces for their culinary traits exerting strong selection pressure on the pathogen population. Landraces were heterogeneous for their resistance, having some highly

resistant plants among susceptible ones (El Amil *et al*., in preparation). The number of detected MLGs in this study was higher than earlier described clonal population (Enjalbert *et al*., 2005). The predominant MLG-34 in this study was found in 120 isolates, 25 Lebanese isolates and 95 Syrian isolates, which represented about half the population. Most of the MLGs were closely related to PstS2 aggressive strain. Comparing the dominant MLG-34 and MLG-99 corresponding to PstS2 strain in the study of Ali *et al.* (2014a), the two MLGs differed by only one allele on four heterozygous SSR markers but shared the WU12 marker that marked the aggressive signature (Ali *et al*., 2014a). The most related MLGs to MLG-99 were detected in the Syrian population differing by maximum three SSR markers with the same set of microsatellites. This divergence from MLG-99 had been detected resulting in a rapid shift and in emergence of new variants of the PstS2. A similar result was observed major shift revealing in the United Kingdom in 2013 where the *Pst* population structure showed four distinct lineages that had replaced the previous *Pst* population (Hubbard *et al*., 2015). Furthermore, new variants of Ug-99 stem rust, the invasive race since 1999, had been reported and became dominant in Africa in 2010 (Wolday *et al*., 2011). In the study we separated *Pst* genotypes collected from bread wheat, durum wheat, Triticale and volunteer and we found no differentiation between the host type.

Comparing our studies and previous ones, high pathotype diversity was detected in the region with a clonal and clear subdivision population in Lebanon and Syria, and the spread of dominant MLG closely related to those of the PstS1/S2 aggressive strains spread worldwide. This information could be useful for the region for better integrating disease management. Cultivation of cultivars with different resistance genes would prevent the selection and uniform spread of a specific pathotype in the region. Diversification in term of resistance genes over time could help in retarding emergence of new virulences. Along with durable deployment of resistance genes in breeding programs, population genetic structure of *Pst*, and combination of both types of resistance, seedling and adult plant resistance, confer better protection and considered as best strategy for durable resistance (de Vallavieille-Pope *et al*., 2012).

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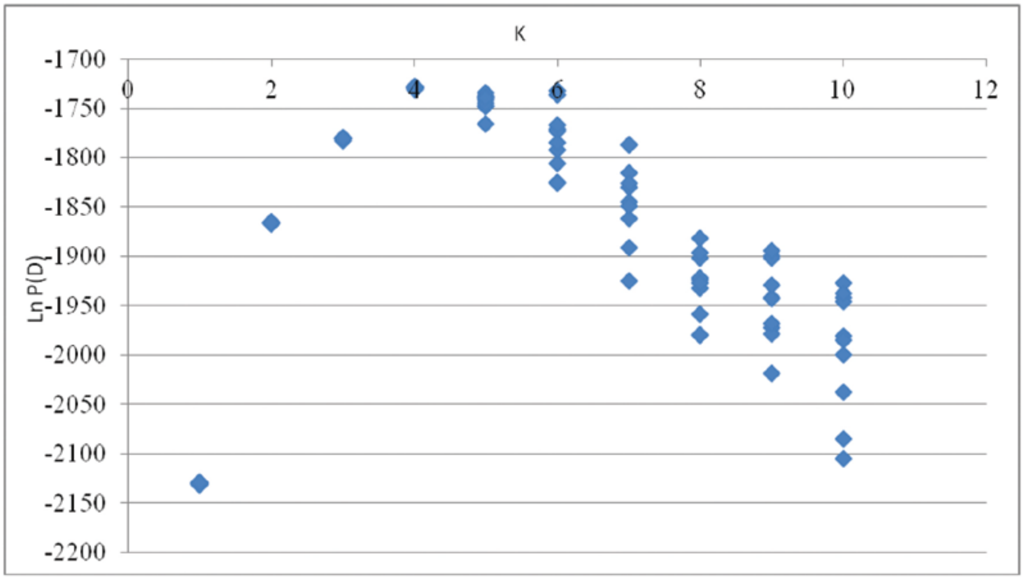
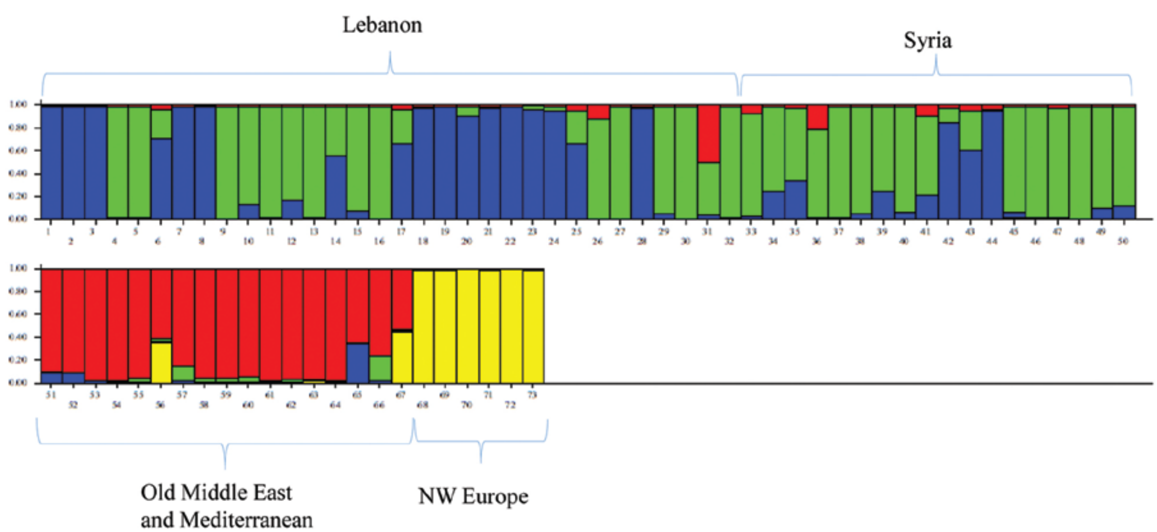


Supplementary figure1: Distribution of mean Ln P(D) values estimated under STRUCTURE software for *Puccinia striiformis* f. sp. *tritici* populations, suggesting K =4 as the optimal number of populations (K) following Evanno *et al*. (2005).



Supplementary figure 2: Clustering of 362 *Puccinia striiformis* f. sp. *tritici* isolates collected in Lebanon and Syria in 2010-2011 (50 MLG), in Middle East and Mediterranean in 2004-2005 (17 MLG), and in North-Western Europe in 2008-2009 (6 MLG), using STRUCTURE at K=4 following the method of Evanno *et al*. (2005).

Supplementary table 1: Linkage disequilibrium test generated on several pairs of loci of SSR markers by using GENPOP 4.0 software.

Locus pair	Chi2	df	P-value significance
RJN5&RJO21	0	2	1
RJN6&RJO21	0	2	1
RJN3&RJO21	0	2	1
RJN5&RJN8	5.327	4	0.255
RJN6&RJN8	3.605	4	0.461
RJN13&RJN8	5.582	2	0.061
RJN3&RJN8	5.643	4	0.227
RJO21&RJN8	0	2	1
RJN5&RJN10	0	4	1
RJN6&RJN10	9.658	4	0.046
RJN13&RJN10	0	2	1
RJN3&RJN10	0	4	1
RJO21&RJN10	0	2	1
RJN8&RJN10	0	4	1
RJN5&RJO24	4.268	4	0.37
RJN6&RJO24	8.819	4	0.065
RJN13&RJO24	3.39	2	0.183
RJN3&RJO24	3.362	4	0.499
RJO21&RJO24	0	2	1
RJN8&RJO24	0	4	1
RJN10&RJO24	14.218	4	0.006
RJN5&RJO4	9.3	4	0.054
RJN6&RJO4	8.299	4	0.081
RJN13&RJO4	8.143	2	0.017
RJN3&RJO4	8.241	4	0.083
RJO21&RJO4	0	2	1
RJN8&RJO4	0	4	1
RJN10&RJO4	17.147	4	0.001
RJN5&RJN11	0	4	1
RJN6&RJN11	0	4	1
RJN13&RJN11	0	2	1
RJN3&RJN11	0	4	1
RJO21&RJN11	0	2	1
RJN8&RJN11	0	4	1
RJN10&RJN11	0	4	1
RJO24&RJN11	3.323	4	0.505
RJO4&RJN11	1.643	4	0.801
RJN5&RJN2	9.319	4	0.053
RJN6&RJN2	1.212	4	0.876
RJN13&RJN2	7.963	2	0.018
RJN3&RJN2	7.847	2	0.097
RJO21&RJN2	0	2	1
RJN8&RJN2	0	4	1
RJN10&RJN2	4.837	4	0.304

Continued Supplemantry table 1:

RJN11&RJN2	1.53	4	0.821
RJN5&RJN2	0	2	1
RJN6&WU6	0	2	1
RJN3&WU6	0	2	1
RJO21&WU6	0	2	1
RJN8&WU6	0	2	1
RJN10&WU6	0	2	1
RJO24&WU6	4.921	2	0.085
RJO4&WU6	5.98	2	0.05
RJN11&WU6	0	2	1
RJN2&WU6	8.93	2	0.011
RJN5&WU6	10.359	4	0.034
RJN6&WU12	2.29	4	0.682
RJN13&WU12	8.592	2	0.013
RJN3&WU12	8.667	2	0.069
RJO21&WU12	0	2	1
RJN8&WU12	0	4	1
RJN10&WU12	0.994	4	0.91
RJN11&WU12	1.455	4	0.91
WU6&WU12	6.506	2	0.038
RJN5&RJO27	0	4	1
RJN6&RJO27	3.622	4	0.459
RJN13&RJO27	0	2	1
RJN3&RJO27	0	4	1
RJO21&RJO27	0	2	1
RJN8&RJO27	0	4	1
RJN10&RJO27	0	4	1
RJO24&RJO27	7.91	4	0.094
RJO4&RJO27	0	4	1
RJN11&RJO27	0	4	1
RJN2&RJO27	0	4	1
WU6&RJO27	0	2	1
WU12&RJO27	6.493	4	0.165

Supplementary table 2: Pairwise divergence of *Puccinia striiformis* f. sp. *tritici* isolates from several locations of Lebanon and Syria for Fst (upper diagonal) and Nei genetic distance (lower diagonal) based on 20 microsatellite markers using GenAlEx software.

		TalAmara	Terbol	Kfardan	Aleppo	Raqa	Kamishly	Hassakeh
	TalAmara	0.000	0.01	0.011	0.017	0.027	0.008	0.026
	Terbol	0.010	0.000	0.029	0.039	0.053	0.019	0.05
Lebanon	Kfardan	0.010	0.033	0.000	0.007	0.013	0.021	0.011
	Aleppo	0.016	0.046	0.003	0.000	0.003	0.018	0.002
	Raqa	0.024	0.062	0.008	0.003	0.000	0.029	0.001
	Kamishly	0.009	0.022	0.019	0.019	0.027	0.000	0.027
Syria	Hassakeh	0.021	0.057	0.006	0.001	0.001	0.024	0.000

Supplementary table 3: Pairwise divergence of *Puccinia striiformi*s f. sp. *tritici* isolates from subpopulations 1 and 2 and admixtures (identified under STRUCTURE), Middle East and Mediterranean, and North West Europe genetic groups for Fst (upper diagonal) and Nei genetic distance (lower diagonal) based on 20 microsatellite markers using GenAlEx software.

	Subpopulation 1	Subpopulation 2	REFMDME[a]	REFNWE[b]
Subpopulation 1	0.000	0.102	0.083	0.293
Subpopulation 2	0.138	0.000	0.051	0.292
REFMDME[a]	0.133	0.069	0.000	0.249
REFNWE[b]	0.527	0.486	0.426	0.000

[a] References isolates corresponding to the old Middle East and Mediterranean genetic group (Ali *et al*., 2014b).

[b] References isolates corresponding to the North Western European genetic group (Ali *et al*., 2014b).

CHAPTER 3

Effect of the temperature on aggressiveness components, infection efficiency and latency period, on *Puccinia striiformis* f. sp. *tritici* in the Middle East

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Introduction

It is now generally accepted that average temperatures are rising globally, with rates of change increasing towards the poles, and increasing average temperatures associated with more frequent extreme temperature events (IPCC 2007). These changes in environmental conditions will lead to selection on living organisms, which, in order to persist, will need to migrate, tolerate or adapt to locally changed conditions. Range shifts to higher latitudes or elevations are already underway (Parmesan 2006) including for crop pests (Bebber *et al.* 2013). The ability to tolerate climate change or adapt to it while staying in the same site depends the breadth of genetic variation for temperature tolerance. Variation within and among populations has been observed for a large range of organisms including insects, plants and fungi (Sørensen *et al.* 2001; Wahid *et al.* 2007; Fallis *et al.* 2011; Angert *et al.* 2011; Mboup *et al.* 2012; Stefansson *et al.* 2013; Sternberg & Thomas 2014), suggesting that adaptation to changing conditions should be possible (Reusch & Wood 2007).

Plant diseases will, therefore, pose an ever-increasing ecological and evolutionary challenge. In addition to the perpetual adaptation of diseases to host resistance in natural (Tack *et al.* 2012) and agricultural (Kiyosawa 1982) systems, climate change may lead to range shifts with new diseases invading previously untouched areas (Evans *et al.* 2008; Luck *et al.* 2011; Shaw & Osborne 2011). Furthermore, pests and diseases may adapt to changing conditions, for example increasing the breadth of their temperature tolerance or shifting their temperature optima.

Wheat stripe (yellow) rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), provides one such example. Historically considered a disease of temperate zones, *Pst* was only sporadically recovered from hotter grain production areas such as Western Australia and the mid-west of North America, presumably because it was unable to tolerate the high temperatures (Wellings 2011). However, a new strain, referred to as PstS1 (Hovmøller *et al*. 2008), caused severe and widespread epidemics in the North American mid-west in 2000 (Chen *et al.* 2002). An almost identical strain was recovered from Western Australian in 2002 and from Eastern Australia in 2003 , where it also led to major epidemics (Wellings 2011). Evidence as to whether the aggressive strains responsible for these epidemics are adapted to high temperatures remains somewhat equivocal (Milus *et al.* 2009; Loladze *et al.* 2014). Milus *et al*. (2009) showed adaptation to high temperature of *Pst* isolates post 2000 from North America, Eritrea and Denmark compared to American isolates pre-2000, whereas comparison of Australian isolates pre and post-2002 did not show adaptation to high temperature. On the other hand, in France, there is clear evidence for local adaptation to temperature conditions in *Pst*. Southern strains outperform northern ones at high temperatures and northern strains outperform southern ones at low temperatures under controlled experimental conditions (Mboup *et al.* 2012).

Which strain will occur in a particular region depends not only on its ability to thrive under local climatic conditions, but also on the resistance structure of available hosts. Indeed, in the example cited above (Mboup *et al.* 2012), southern French strains outperformed northern strains under all field conditions, even northern ones, when susceptible hosts were provided. Hence, disease emergence will be conditioned by local host availability as well as climatic adaptation of the pathogen. The Middle East and the Mediterranean basin represent a cradle of agriculture and a centre of wheat diversity. Indeed we have found a number of uncharacterised resistance phenotypes in landraces that are grown throughout the Middle East (El Amil *et al*. in prep) and many of these landraces segregated for resistance, demonstrating that they are variable and heterozygous for these novel resistance types. In the wheat-growing areas of North America, North Western Europe and Australia, on the other hand, a single homogeneous variety is grown over very large scales. The wheat cultural practices in the Middle East therefore probably exert a more diversified selection regime on wheat pathogens than is experienced in wheat-growing zones of North America and Australia. In addition, the climatic conditions vary over small geographical regions, from

dry-Mediterranean climates to cooler wetter conditions in the highlands. We therefore set out to test a set of *Pst* isolates from throughout the Middle East, collected from warmer and cooler sites from each region, for their performance under contrasting cool and warm temperature regimes. This will provide a better understanding of the breadth of temperature adaptation in *Pst* populations from this region and thus how rapidly we can expect *Pst* to track global environmental change. Since temperature adaptation had already been observed in some French isolates of this fungus (Mboup *et al.* 2012), we predict that fungal isolates from warmer regions would perform better than those from cooler regions under warm experimental penetration and incubation temperatures. We carried out a fully factorial experiment to test the relative importance of penetration and incubation temperatures and their interactions on two important life history characters of this plant pathogen – infection efficiency and latency period or time until sporulation. We also tested whether isolates with genetic markers that ally them to two aggressive strains that had caused recent epidemics, here referred to as PstS1 and PstS2, thought to be adapted to high temperatures (Milus *et al.* 2009), perform better under high temperature conditions. Finally we test whether there is a correlation between phenotypic and genetic distance, the latter calculated based on neutral markers.

Materials and Methods

Details of the *Pst* isolates used in this study are given in (Table 1) and briefly summarized here. Of the 30, four were reference isolates with previously characterized temperature specificities. These were included to make sure that the experimental conditions gave results comparable with previous ones and to position the other isolates with respect to these known isolates but they were not included in the analyses of temperature adaptation. The reference isolates include three isolates previously characterized as warm-adapted (PstS2: DK66/02, PstS1: ET02/10, and Fr6) (Milus *et al.* 2009; Mboup *et al.* 2012) and one cold-adapted isolate (Fr232) (de Vallavieille-Pope *et al.* 1995, 2002). Of the other 26 isolates, 18 were obtained from the collections of the Institut National de Recherche Agronomique, BIOGER, France (10), Global Rust Reference Center, Aarhus University, Denmark (6) and the International Center of Agricultural Research in Dry

Table 1. Set of 26 isolates of *Puccinia striiformis* f. sp. *tritici* selected in the Middle East region (wild isolates) and 4 reference isolates, the climate of region of sampling, year of collection, virulence profiles and genotypes.

Study code	Isolate type	Country	Location	Latitude/longitude	Elevation (m)	Climate[a]	Year	Wheat Type[b]	SCAR[c]
DZ283	Wild	Algeria	Matmort	35°23'N/01°51'E	876	Cold	2006	BW	Other
CY001	Wild	Cyprus	Athalassa	35°08'N/33°24'E	141	Warm	2005	BW	PstS1
CY005	Wild	Cyprus	Xylotymbou	35°01'N/33°45'E	55	Warm	2006	BW	PstS2
CY007	Wild	Cyprus	Xylotymbou	35°01'N/33°45'E	55	Warm	2006	BW	PstS2
IR183	Wild	Iran	Dezful	32°22'N/48°24'E	137	Warm	2005	BW	PstS2
IR90/63	Wild	Iran	Zarghan	29°46'N/52°43'E	1593	Cold	2011	BW	PstS2
IR91/50	Wild	Iran	Ardebil	38°15'N/48°17'E	1346	Cold	2011	BW	PstS2
LB442	Wild	Lebanon	Sariin	33°91'N/36°06'E	1021	Cold	2006	BW	PstS2
LB446	Wild	Lebanon	Sariin	33°88'N/36°08'E	1006	Cold	2006	BW	Other
LB6E0	Wild	Lebanon	Deir Zanoun	33°77'N/35°93'E	885	Cold	2006	BW	PstS1
LB13	Wild	Lebanon	Tal Abbas	34°34'N/36°04'E	46	Warm	2012	BW *cv.*Lee	Other
LB15	Wild	Lebanon	Kounin	33°08'N/35°26'E	634	Cold	2012	BW	PstS2
SP10A	Wild	Spain	Jerez	36°41'N/06°07'W	45	Warm	2006	BW	PstS2
SY06/09	Wild	Syria	Tal Hadya	35°57'N/36°56'E	284	Warm	2009	BW	Other
SY11/09	Wild	Syria	Tal Hadya	35°57'N/36°56'E	284	Warm	2009	BW	PstS2
SY04/09	Wild	Syria	Al Gab Alziara	34°43'N/36°43'E	476	Cold	2009	BW	PstS2
SY03/10	Wild	Syria	Hassakeh	36°35'N/40°35'E	417	Cold	2010	BW	PstS2
SY01/10	Wild	Syria	Tal Hadya	35°57'N/36°56'E	284	Warm	2011	BW	Other
SY02/10	Wild	Syria	Tal Hadya	35°57'N/36°56'E	284	Warm	2011	BW *cv.*Cham8	PstS2
Tu253	Wild	Tunisia	Krib	36°16'N /8°58'E	300	Warm	2005	BW	PstS2
TK34/11	Wild	Turkey	Adana	37°00'N/35°19'E	14	Warm	2011	BW *cv.*Irnerio	PstS2
TK58/11	Wild	Turkey	Ankara-Haymana	39°26'N/32°29'E	1076	Cold	2011	BW TP981	PstS2
TK11	Wild	Turkey	Urfa-Gundas	36°43'N/38°48'E	378	Warm	2012	BW *cv.*Opata	Other
TKN45	Wild	Turkey	Eskisehir	39°46'N/30°31'E	794	Cold	2012	BW	PstS1
LB75061	Wild	Lebanon	Tal Amara	33°80'N/35°82'E	905	Cold	1975	Unknown	Other
LB74015	Wild	Lebanon	Houch Sneid	34°54'N/36°45'E	1000	Cold	1974	Unknown	Other
DK66/02	Reference[d]	Denmark	Denmark	55°23'N/10°24'E	19	Warm	2002	Unknown	PstS2
ET02/10	Reference[d]	Ethiopia	Ethiopia	Unknown	Unknown	Warm	2007	Unknown	PstS1
Fr6/J8617	Reference[e]	France	La Bâtie-Rolland	44°33'N/4°51'E	156	Warm	1986	DW *cv.*Prinqual	Other
Fr232/J89108	Reference[f]	France	Mouflers	50°02'N/02°03'E	41	Cold	1989	BW *cv.*Thésée	Other

[a]The choice of isolates made from different climate regions based on the annual mean temperature: warm, cold and unknown for the non distinguished region.
[b]BW and DW correspond to bread and durum wheat, respectively
[c] a SCAR marker developed at Aarhus University (Hovmøller *et al.*, 2008) permitted assignment to the strains PstS1 or PstS2
[d]Milus *et al.*, 2009
[e]Mboup *et al.*, 2013
[f]de Vallavieille-Pope *et al.*, 1995, 2002

Areas, Syria (2). An additional 8 isolates were collected from field surveys of bread wheat growing in coastal and mountainous areas of Lebanon, Syria and Turkey during the cropping season 2011-2012. Isolates were chosen to maximize pathotype diversity and geographic origin, and to have some strains from cold and some from warm conditions. We classified local conditions based on latitude, elevation and mean temperatures from February to May, the growing season of wheat in the Mediterranean region. This enabled us to test for local adaptation to temperature conditions, i.e. whether isolates from warm areas perform better under warm conditions.

The virulence profiles of all isolates were tested at INRA, Versailles as described in de Vallavieille-Pope *et al*. (2012). All isolates were genotyped for 20 microsatellite markers (Ali *et al.* 2011) at INRA Grignon and for a SCAR marker at Aarhus University (Hovmøller *et al.* 2011), the latter which permitted us to determine, for each strain, whether it belonged to the aggressive strains PstS1 or PstS2 (Table 2).

The temperature experiment was carried out at INRA, Versailles, in a high containment spore-proof climate controlled chamber with level 3 security to prevent the escape of foreign spores. All plants were cultivated at 16^0C-19^0C with 16-h photoperiod from natural and supplemental light at 200 $\mu E/m^2/s^1$ before experimental temperature treatments. Before inoculation the photoperiod was modified to give plants 16 hours of light just prior to the inoculation treatment, followed by 24 hours at 100% humidity in the dark post-inoculation, at 8^0C for spore multiplication (de Vallavieille-Pope *et al.* 2002) and varying temperatures for the experiments. To enhance spore production and to prevent leaf elongation each pot was treated with 20 ml of maleic hydrazide (0.25 g/liter) solution when seedlings were at 1-cm tall. To ensure genetic purity, a single lesion of each *Pst* isolate was collected after initial inoculation at low spore numbers. This lesion was rubbed onto 10 seedlings of two susceptible cultivars (Victo and Michigan Amber) growing mixed in a single pot and subsequently enclosed in a cellophane bag. After 14-17 days we collected as many spores as possible by tapping them onto the cellophane bag and dried them in a desiccator filled with Silicagel at 4^0C for 3 days. These spores were suspended in mineral oil Soltrol 170 (Chevron-Phillips Chemical Co., Houston) and sprayed onto 3 or 4 pots of seedlings pretreated as above for a first round of multiplication that was followed by an identical second round of multiplication. Then spores were collected, dried and stored in small vials at -80^0C. A third multiplication was done just prior to each experiment and spores were harvested and dried as above.

Table 2. Virulence profiles, SCAR markers and genotypic groups of 26 isolates of *Puccinia striiformis* f. sp. *tritici* selected in the Middle East region (wild isolates) and 4 reference isolates

Study code								Pathotype[a]											SCAR[b]	Genetic group[c]
	1	2	3	4	5	6	7	8	9	10	15	17	25	27	32	SD	SP	Su		
DZ283	1	2	3	4	-	6	-	-	9	-	-	17	25	-	-	SD	-	Su	Other	NW European
CY001	-	2	-	4	-	6	7	8	9	-	-	-	25	-	-	-	-	-	PstS1	Middle East-East Africa
CY005	-	2	-	-	-	6	7	8	-	-	-	-	25	-	-	-	-	-	PstS2	Middle East-East Africa
CY007	-	2	-	-	-	6	7	8	-	-	-	-	-	-	-	-	-	-	PstS2	Middle East-East Africa
IR183	-	2	-	-	-	6	7	8	9	-	-	-	25	27	-	(SD)	-	-	PstS2	Middle East-East Africa
IR90/63	-	2	-	-	-	6	7	8	9	-	-	-	25	-	-	-	-	-	PstS2	Middle East-East Africa
IR91/50	-	2	-	-	-	6	7	8	9	-	-	-	25	-	-	-	-	-	PstS2	Middle East-East Africa
LB442	-	2	-	-	-	6	7	8	9	-	-	-	25	27	-	-	-	-	PstS2	Middle East-East Africa
LB446	-	2	-	-	-	6	7	8	-	-	-	-	-	-	-	-	-	-	Other	Middle East-East Africa
LB6E0	-	2	-	-	-	6	7	8	9	-	-	-	-	-	-	-	-	-	PstS1	Middle East-East Africa
LB13	-	2	-	-	-	6	7	8	-	-	-	-	-	-	-	-	-	-	Other	Middle East-East Africa
LB15	-	2	-	-	-	6	7	8	9	-	-	-	25	-	-	-	-	-	PstS2	Middle East-East Africa
SP10A	-	2	-	-	-	6	7	8	-	-	-	-	-	-	-	-	-	-	PstS2	Mediterenean region
SY06/09	-	-	-	-	-	6	7	8	-	-	-	-	-	-	-	-	-	-	Other	Middle East-East Africa
SY11/09	1	2	-	-	-	6	7	8	9	-	-	-	25	-	-	-	-	-	PstS2	Middle East-East Africa
SY04/09	-	(2)	-	-	-	6	7	8	9	-	-	-	-	27	-	(SD)	-	-	PstS2	Middle East-East Africa
SY03/10	-	2	-	-	-	6	7	8	9	-	-	-	-	27	-	-	-	-	PstS2	Middle East-East Africa
SY01/10	-	(2)	-	-	-	6	7	8	9	-	-	-	25	27	-	-	-	-	Other	Middle East-East Africa
SY02/10	-	2	-	-	-	6	7	8	9	-	-	-	-	27	-	-	-	-	PstS2	Middle East-East Africa
Tu253	-	2	-	-	-	6	7	8	-	-	15	-	-	27	-	-	-	-	PstS2	Mediterenean region
TK34/11	-	(2)	-	-	-	6	7	8	9	-	-	-	25	27	-	-	-	-	PstS2	Middle East-East Africa
TK58/11	-	(2)	-	-	-	6	7	8	9	-	-	-	25	-	-	-	-	-	PstS2	Middle East-East Africa
TK11	-	2	-	-	-	6	7	8	-	-	-	-	-	-	-	-	-	-	Other	Middle East-East Africa
TKN45	-	2	-	-	-	6	7	8	9	-	-	-	25	-	-	-	-	-	PstS1	Middle East-East Africa
LB75061	-	2	-	-	-	6	7	8	-	-	-	-	-	-	-	-	-	-	Other	Middle East-East Africa
LB74015	-	2	-	-	-	6	7	8	-	-	-	-	-	-	-	-	-	-	Other	Middle East-East Africa
DK66/02	-	(2)	-	-	-	6	7	8	9	-	-	-	-	-	-	(SD)	-	-	PstS2	Middle East-East Africa
ET02/10	-	2	-	-	-	6	7	8	9	10	-	-	25	27	-	(SD)	-	-	PstS1	Middle East-East Africa
Fr6/J8617	-	2	-	-	-	6	7	8	-	-	-	-	-	-	-	-	-	-	Other	Closed to Middle East
Fr232/J89108	-	2	3	4	-	-	-	-	9	-	-	-	-	-	-	SD	-	Su	Other	NW European

[a]Virulence combinations of yellow rust isolates were determined using the European and world sets of 16 differential varieties (Johnson *et al.*, 1972) and a sub-set of Avocet-based differential lines Avocet *Yr*1, *Yr*2, *Yr*3, *Yr*4, *Yr*5, *Yr*6, *Yr*7, *Yr*8, *Yr*9, *Yr*10, *Yr*15, *Yr*27, *Yr*32 Kalyansona, TP1295, Strubes Dickkopf, Spaldings Prolific, and Suwon 92/Omar were used as source of *Yr*2, *Yr*25, *Yr*SD,*Yr*SP, and YrSu, respectively. Virulence given in brackets corresponded to intermediate infection types (5-6) on the 0-9 scale, 0 fully resistant, 9 fully susceptible.

[b] a SCAR marker developed at Aarhus University (Hovmøller *et al.*, 2008) permitted assignment to the strains PstS1 or PstS2

[c] The genetic groups based on 20 SSR markers (Ali et *al.*, 2014)

Fifteen seeds of *cv*. Cartago with no known resistance genes to yellow rust, were planted in square pots (7x7x8 cm) filled with standard peat soil, grown in the high confinement glasshouse at 16^0C-19^0C with 16-h photoperiod from natural and supplemental light at 200 $\mu E/m^2/s^1$ and half of the pots, those for the latency period tests, were treated with maleic hydrazide as above. Pots were thinned to ten homogenous seedlings.

We assessed infection efficiency and latency period of all isolates at two different dew temperatures (5^0C and 20^0C) and two different incubation temperatures (10^0C -15^0C and 16^0C-25^0C). Using pots at the two-leaf stage, we inoculated 32 pots with 0.5 mg of urediniospores suspended in 300 mL of mineral oil Soltrol 170 of each fungal isolate. After 10 minutes for oil evaporation at room temperature each tray of 16 pots, representing a treatment combination, was placed in a wet plastic bag for 24 h with 100% relative humidity at either 5^0C or 20^0C dew temperature, in the dark to permit penetration of the fungus. Subsequently, these 16 pots were split into two different climate chambers with different temperature regimes (daylight 300 $\mu E/m^2/s^1$, during 16 h at 15^0C and darkness for 8 h at 10^0C) and (daylight 300 $\mu E/m^2/s^1$, during 16 h at 25^0C light and darkness for 8 h at 16^0C). We removed the second and third leaves of all the seedlings 7 days after inoculation. Seven to 10 days after inoculation, as soon as the first chloroses were visible, we counted chloroses on the first leaf of 4 pots per dew temperature and incubation temperature combination per isolate to determine infection efficiency (IE). IE, which represents the proportion of deposited spores successfully infecting the leaf and causing symptom development, was calculated as the number of chloroses divided by the leaf surface (length x width, mm^2) and the density of deposited spores/mm^2. After counting chloroses we discarded these pots.

Two measures of latency period are commonly used: LP1 is the time interval from inoculation to the first appearance of spores in new uredinia breaking the leaf epidermis (Jeffrey *et al*. 1962; Miller *et al.* 1998) and (LP50) is the time needed for half of the final number of lesions to sporulate (Knott & Mundt 1991; Flier & Turkensteen 1999) or to show sporulation structures (Johnson 1980; Tomerlin *et al.* 1983). For the remaining 4 pots per dew temperature and incubation temperature combination per isolate, starting the 8th day post-inoculation, we counted all sporulating lesions on each inoculated leaf daily. We marked each sporulating lesion with an ink pen on the leaves to be able to identify newly sporulating lesions, and continued these observations on each pot until the day after which no additional symptom development was observed on any plant in that pot. We originally planned to estimate spore production but climate chamber conditions appeared not appropriate for proper

spore development. All our plants became wilted and chlorotic and it was impossible to collect spores.
This experiment was replicated three times in March, April and June 2013 for a total of 2880 pots.

Statistical analysis

We worked with the mean values for our variables per pot. Using only the 26 isolates from the Middle East and Mediterranean region we tested whether infection efficiency, following natural log transformation to homogenize variances, and latency, both LP1 and LP50, varied among dew and incubation temperature combinations. We tested also whether this depended on isolate origin, i.e., from cold versus warm conditions for the Middle Eastern and Mediterranean samples, or on the SCAR/SSR group determined from genotyping. We used a factorial, hierarchical ANOVA followed with Tukey multiple comparison tests where relevant. Dew and incubation temperatures were analyzed in a factorial manner. Isolate was treated as a random factor nested within isolate origin (i.e. whether from cold or warm conditions, or SCAR/SSR group). Analyses were carried out using the JMP statistical package (SAS2009).

To visualize patterns of phenotypic similarity we carried out a principal component analysis (PCA) using data from all strains, including the reference isolates and those from outside the Middle East and Mediterranean regions, using XLSTAT (XLSTAT version 2012.6.01). This analysis provided coordinates for the position of each isolate in phenotypic space based on the first two principal components (PCA1, PCA2). We compared pairwise genetic distances, based on the 20 microsatellite data, with pairwise phenotypic distances on the PCA1 and PCA2 plot between isolates, using a Mantel test calculated in GENEPOP, to test whether genetic and phenotypic distances were correlated.

Results

Temperature adaptation to climatic conditions of origin

Infection efficiency was very sensitive to variation in dew temperature, with very low infection efficiency at high dew temperature. IE for the different *Pst* isolates varied from 7.56% to 15.23% under the cold/warm regime; from 5.65% to 12.8% under cold/cold regime; from 0.04% to 0.935% under warm/cold regime and from 0.05% to 0.875% under warm/warm regime.

There was a non-significant tendency for higher infection efficiency at warm incubation temperature (Table 3, Figure 1). *Pst* isolates that had experienced cold dew temperature post inoculation had higher infection efficiency when subsequently incubated at warm temperatures but *Pst* isolates that had experienced warm dew temperature had higher infection efficiency when subsequently incubated at cold temperature, shown by the significant dew temperature * incubation temperature interaction (Table 3, Fig 1). We found no evidence of local adaptation to climatic conditions in infection efficiency. Infection efficiency did not vary significantly between isolates originating from warm versus cold climates, either as a main effect or in any interactions.

Latency period, both until first sporulation or until 50% sporulation, on the other hand, showed patterns of local adaptation. The two variables gave very similar results, so we chose to present the analyses for latency period until first sporulation throughout. Latency period was almost 18 hours shorter on average at low than at high dew temperature but over 70 hours shorter at high than at low incubation temperature. There was a significant interaction between the two, such that warm dew temperatures retarded sporulation more when incubation temperatures were warm compared to cold (Table 4, Figure 2). Though latency period did not vary significantly with climate of origin either as a main effect or in two-way interactions with the experimental dew or incubation temperature regime, there was a significant three-way interaction (Table 4).

Table 3. Infection Efficiency (IE): Fully factorial mixed-model hierarchical ANOVA analysing the 26 Middle Eastern and Mediterranean isolates, testing the effect of cold versus warm climate of origin, experimental cold (5°C) and warm (20°C) dew temperatures and incubation temperatures (10°C night/15°C and 16/25°C day), with isolates treated as a random factor nested within climate of origin, and controlling for the three experimental blocks. R^2 for this analysis = 0.9255.

Fixed Effects Tests

Source of variation	Numerator df	Denominator df	F Ratio	Probability
Climate of origin	1	24.19	0.0003	0.985
Dew temperature	1	26.1	2854.39	<0.0001
Incubation temperature	1	23.95	3.75	0.065
Dew temperature*Incubation temperature	1	23.84	15.88	0.0006
Dew temperature * Climate of origin	1	23.86	0.03	0.87
Incubation temperature* Climate of origin	1	23.95	0.38	0.54
Dew temperature*Incubation temperature *Climate of origin	1	23.84	0.09	0.77
Block	2	933.7	44.30	<0.0001

Random Effects Tests

	Variance Ratio	Variance Component	% total variance
Isolate(Climate of Origin)	0.0062324	0.000498	0.512
Dew temperature * Isolate(Climate of origin)	0.1388559	0.011096	11.402
Incubation temperature * Isolate(Climate of origin)	0.0180679	0.0014438	1.484
Dew temperature * Incubation temperature * Isolate(Climate of origin)	0.0546584	0.0043678	4.488
Residual		0.0799101	82.114

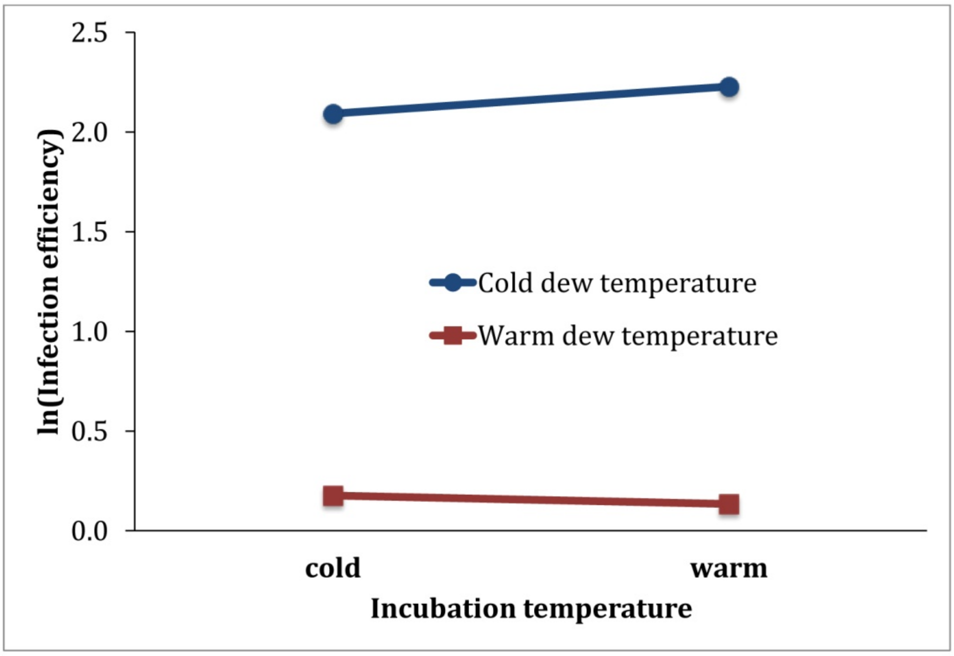

Figure 1. Infection efficiency for the four combinations of cold and warm dew and incubation temperatures. Non-parallel lines illustrate the significant dew temperature * incubation temperature interaction in Table 3.

When incubated at cold temperature, isolates originating from cold and warm climates responded similarly to variation in dew temperature. This was not the case when incubated at warm temperature. Under the warm incubation regime *Pst* isolates from cold climatic conditions took significantly longer to sporulate when they had experienced a warm than a cold dew temperature. Those from a warm climate did not differ in latency time between the two dew temperatures when incubated under warm conditions. Overall, warm dew temperatures retarded sporulation, but this effect was far less marked for isolates from warm climates when incubated under warm conditions, with isolates from warm climates being more resistance to the retarding effects of warm dew temperatures (Table 4, Figure 3)

Temperature adaptation of isolates belonging to different genetic groups

Here we tested whether isolates belonging to the genetic groups characterised as new, highly aggressive and adapted to high temperatures indeed performed better, showing higher infection efficiency and shorter latency periods, under warm versus cold experimental conditions. For infection efficiency, as for the previous analysis that compared isolates from cold versus warm climates of origin, infection efficiency was higher at low dew temperature and there was a significant interaction between dew and incubation temperature. The results resemble closely those presented in Table 3, and are therefore not shown. Genetic group to which the isolates belonged, i.e. the aggressive PstS1 or PstS2 versus other genetic groups, did not explain significant variation in Infection Efficiency, either as a main effect of in any interactions. For latency period, both until first sporulation or until 50% sporulation, however, we found a significant interaction between genetic group and incubation temperature (Table 5, Figure 4). The isolates identified as PstS2 sporulated more rapidly than did the other two groups at warm incubation temperatures, which supports the idea that this genetic group is warm-adapted.

Correlation between genetic and phenotypic distance

The principal component analysis (PCA) provided an overall summary of results of the two epidemiological parameters (infection efficiency and latent period 50%) under the 4 temperature regimes for the 30 *Pst* isolates. The first two components of the PCA accounted for 61% of the original variation. The principal component analysis based on data on infection efficiency and the latency 50% measures under the four different dew- and incubation-temperature combinations did not generate clear groups either of isolates coming from warm versus cold climatic conditions or of the different genetic groups.

Table 4. Latency period to first sporulation (LP1): Fully factorial mixed-model hierarchical ANOVA analysing the 26 Middle Eastern and Mediterranean isolates, testing the effect of cold versus warm climate of origin, experimental cold (5°C) and warm (20°C) dew temperatures and incubation temperatures (10°C night/15°C and 16/25°C day), with isolates treated as a random factor nested within climate of origin, and controlling for the three experimental blocks. R^2 for this analysis = 0.6932.

Fixed Effects Tests

Source of variation	Numerator df	Denominator df	F Ratio	Probability
Climate of origin	1	22.87	0.24	0.63
Dew temperature	1	27.2	60.45	<0.0001
Incubation temperature	1	26.21	1310.34	<0.0001
Dew temperature*Incubation temperature	1	24.74	16.61	0.0004
Dew temperature * Climate of origin	1	25.99	0.45	0.50
Incubation temperature* Climate of origin	1	26.12	1.22	0.28
Dew temperature*Incubation temperature *Climate of origin	1	24.64	6.73	0.016
Block	2	879.2	74.58	<0.0001

Random Effects Tests

	Variance Ratio	Variance Component	% total variance
Isolate(Climate of Origin)	0.0512095	34.385105	4.656
Dew temperature * Isolate(Climate of origin)	0.0300714	20.191751	2.734
Incubation temperature * Isolate(Climate of origin)	0.008641	5.8020935	0.786
Dew temperature * Incubation temperature * Isolate(Climate of origin)	0.0099325	6.6692685	0.903
Residual		671.45937	90.921

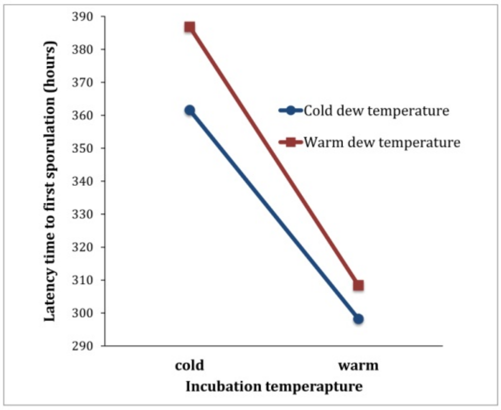

Figure 2 Latency time to first sporulation for the four combinations of cold and warm dew and incubation temperatures. Non-parallel lines illustrate the significant dew temperature * incubation temperature interaction in Table 4.

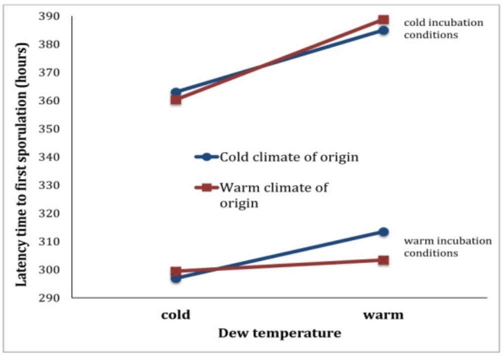

Figure 3. Latency period until first sporulation for the four combinations of cold and warm dew temperatures and cold and warm incubation temperatures plotted separately for isolates from cold versus warm climates of origin. Pst development was much faster when incubation conditions were warm. Non-parallel and crossing lines illustrate the significant dew temperature * incubation temperature * climate of origin interaction in Table 4.

(Figure 5) shows the plot of isolates, identified by their climate of origin and their genetic group, on the first two principal components. According the Eigen vectors of principal component (PC) analysis for the eight variables (infection efficiency at low and high penetration temperature followed by high and low incubation temperature, and latent period at low and high penetration temperature followed by high and low incubation temperature), the representation of the two first PCs showed the PC1 axis which corresponded to the most variation detected, classified isolates for the LP values, being long in the positive direction and short in the other direction.

PC2 classified isolates with high IE values at 20°C and intermediate-high value at 5^{0}C in the right upper quadrant of the Figure 5, and low IE at 20°C and intermediate-low IE at 5°C. In this quadrant the isolates have also long LP at 5°C and 20°C/25 and intermediate-short LP at 20°C /15°C.

In the opposite left low quadrant, the isolates have low IE at 20°C and intermediate-low IE at 5°C. In this quadrant isolates have also short LP at 5°C and at 20°C/25°C and intermediate –long LP at 20°C/15°C. In the upper left quadrant, the isolates have low IE at 5°C and intermediate-high IE at 20°C, furthermore they showed short LP at 5 and 20°C. In the opposite low right quadrant, the isolates have high IE at 5°C, intermediate-low IE at 20°C, and long LP at 5 and 20°C. The four reference isolates corresponded to what was expected for their adaptation to warm and cold conditions. Fr232 the cold-adapted reference isolate (Mboup *et al.* 2012) was in the bottom right quadrant, corresponding to high IE at low dew temperature and long latent period. The two reference isolates for warm temperature, French Fr6 isolate (Mboup *et al.* 2012) and the DK66/02 PstS2 isolate (Milus *et al.* 2009) were in the left upper quadrant, corresponding to low IE at 5°C, intermediate-high IE at 20°C and short LP. The aggressive PstS1 reference isolate ET02/10, tolerant to high temperature (Milus *et al.* 2008) was in the upper right quadrant corresponding to high IE at 20°C and long LP. We separated the 26 *Pst* isolates into five groups. Group 1 in the upper right quadrant includes three isolates from cold and three from warm locations, with one isolate PstS2, two isolates PstS1, and three isolates with the genotype ‘other’. Group 2 situated in the low right quadrant includes four isolates belonging to cold and one to warm locations. Three isolates were PstS2 and two had the genotype ‘other’ Group 3 situated in the center low right of the PC1-PC2 plan, includes five isolates belonging to warm locations and one to cold location; four were PstS2 and two were

Table 5. Latency period to first sporulation (LP1): Fully factorial mixed-model hierarchical ANOVA analysing the 26 Middle Eastern and Mediterranean isolates, testing the effect of cold versus warm climate of origin, experimental cold (5°C) and warm (20°C) dew temperatures and incubation temperatures (10°C night/15°C and 16/25°C day), with isolates treated as a random factor nested within genetic group Pst1, and controlling for the three experimental blocks. R^2 for this analysis = 0.6932.

Fixed Effects Tests

Source of variation	Numerator df	Denominator df	F Ratio	Probability
Genetic group	2	20.81	2.51	0.11
Dew temperature	1	23.58	41.65	<0.0001
Incubation temperature	1	22.31	1031.46	<0.0001
Dew temperature*Incubation temperature	1	21.51	5.22	0.03
Dew temperature * Genetic group	2	23.71	1.15	0.33
Incubation temperature* Genetic group	2	22.97	3.48	0.048
Dew temperature*Incubation temperature * Genetic group	2	22.12	2.29	0.12
Block	2	880	75.07	<0.0001

Random Effects Tests

	Variance Ratio	Variance Component	% total variance
Isolate(Genetic group)	0.0396505	26.664004	3.669
Dew temperature * Isolate(Genetic group)	0.0196851	13.237734	1.822
Incubation temperature * Isolate(Genetic group)	-0.006396	-4.301313	0.0
Dew temperature * Incubation temperature * Isolate(Genetic group)	0.0213139	14.333072	1.972
Residual		672.47602	92.537

‘other’. Group 4 situated in the center up left of the PC1-PC2 plan included five isolates belonging to cold location; three isolates were PstS2, one PstS1 and one ‘other’.
Group 5 includes five isolates which were all from warm locations and PstS2 genotype. This group 5, having low IE at 20°C and short LP is opposed to Group 1, which has high IE at 20°C and long LP. Group 2 had isolates with efficient IE at 5^0C but long LP and on the opposite the cold isolates from group 3 had low IE at 5°C but short LP. Groups 3 and group 4 had an intermediate behavior comparing with Group 1, 2, and 5.
Nonetheless, when we compared the pairwise phenotypic distance between isolates with their pair-wise genetic distance, we found a significant relationship between phenotypic and genetic distance (Mantel test result from 1000 permutations, p = 0.007). Genetically more distant strains, therefore, were phenotypically more dissimilar in their infection efficiency and latency periods under the four temperature conditions.

Discussion

Under warm incubation temperature, we observed that on average LP was 2 days shorter; this result agrees with Milus *et al.* (2009), who showed that North-American post-2000 isolates had a latent period 3 days shorter than old North-American isolates at 12°C for penetration temperature and at high incubation temperature (12-28^0C). These results were contradictory with Australian pre and post-2002 *Pst* isolates, as higher temperature (penetration temperature of 15°C and incubation temperature of 23°C) generally extended the LP of 1.8 days for all isolates in the same way compared to low penetration temperature of 10°C and low incubation temperature of 17°C (Loladze *et al.* 2014). The percentage of infected leaves was higher for the new pathotype 150E16A+ than the older pathotypes, but temperature adaption was not significant. Mboup *et al*. (2012) demonstrated that Southern French isolates were able to germinated better at 20°C than the Northern isolates, to have a low but positive IE at 20°C for penetration temperature whereas Northern isolates were not able to penetrate at that high temperature. Latent period was 0.9 day shorter and sporulation rate was two time higher at high incubation regime of 22-25°C for southern isolates than Northern isolates.

With our results, IE was more efficient under cold than warm dew temperature revealing that still the cold temperature is considered as primary factor for the penetration suggesting that the cold areas will be more threatened by *Pst* for early disease onset. Unlike for IE, the warm incubation plays a major role in shortening the other aggressiveness component LP. The

suggestion that 18^0C is a high temperature for assessing LP (Milus *et al*. 2006) seems to be uncertain as in our case, we succeeded to assess it at 20^0C but unfortunately spore production assessment was not successful under our climate chamber condition under the ($16/25^0$C) regime.

The two groups 1 and 5 have opposite life trait strategy. The group 4, the closest group to warm adapted temperature, was including an old Lebanese isolate 'other' genotype (LB75061), a new Syrian PstS2 isolate (SY03/10), a new Turkish PstS1 isolate, and a new Lebanese PstS2 isolate and none of PstS2 originated from warm location. Group 1, well adapted to high temperature showing high IE at 20^0C, only one Syrian out of six isolates was PstS2. Among the cold-adapted group 2, four out of the five isolates were originating from cold locations.

The Lebanese isolate having 'other' genotype (LB75061) collected forty years ago, showed adaptation to high temperature and not belonging to the PstS2.

Loladze *et al.* (2014) did not show temperature adaptation within old and new invasive *Pst* Australian isolates rather than variation between individual isolates; this was clearly independent of the categorization into new and old group. Similarly, we observed diversity within the Mediterranean and Middle East isolates for temperature response for IE and LP. There was no clear cut categorization between the warm and cold origin for the *Pst* tested isolates. However, group 2 including IR90/63, IR91/50 and LB74015 were collected from high altitude 1593 m, 1346 m, and 1000 m, respectively were situated close to the cold-adapted reference whereas the group 4 closest to warm-adapted reference included the isolates collected from below 1000 m. Our three *Pst* references adapted to high temperature belong to three different genotypes PstS1, PstS2 and other. Similarly, the Mediterranean and Middle East isolates adapted to high temperature belong to different genotypes.

Latency period is a critical life history character for pathogens and infectious diseases, as it determines the generation time of the pathogen and thereby disease dynamics. Doubling time in an epidemic or outbreak is a direct function of pathogen generation time (Fraser *et al.* 2004), so pathogens with short latency will cause more rapid epidemic spread. Zhang and McDonald (2011) showed that population of *Mycosphaerella graminicola (Zymoseptoria tritici)* originating from warm places perform better for growth rate under the high temperature than those from cold places.

There are two ways of thinking about local adaptation. One is that organisms should perform better when exposed to their habitual rather than a novel environment (the home versus away advantage), the other, that they should outcompete exotic organisms on their home turf (the local versus foreign advantage)(Kawecki & Ebert 2004). Under cold incubation conditions, the times to sporulation of *Pst* isolates collected from cold or warm climates were undistinguishable from one another, both when they had experienced cold and when they had experienced warm dew temperatures. In all cases latency was long. When incubated at warm temperatures, on the other hand, first, latency was much shorter. Second, though isolates collected from cold or warm climates were undistinguishable from one another when they had experienced cold dew temperatures, after a warm dew temperature treatment, isolates from warm climatic conditions clearly out-performed those from cold origins. We note that, though the latency period was slightly shorter, even for the isolates from warm climatic conditions, for the combination of cold dew temperature and warm incubation temperature, that at warm dew and incubation temperatures isolates from warm climatic conditions had a large relative advantage over those from cold conditions. Therefore this is a case when our isolates of warm climatic origin can be shown to be locally adapted to their climatic conditions following the "local versus foreign advantage" criterion, but not for the "home versus away advantage" criterion.

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GENERAL DISCUSSION

General Discussion

The present study was designed to describe the diversity of *Puccinia striiformis* f. sp. *tritici* and its host in the Middle East especially in Lebanon and Syria. The Middle East located in Near East Fertile Crescent is considered the cradle of agriculture and well known for the richness for wheat and its wild relatives (Heun *et al.*, 1997). The region experienced many recurrent epidemics (Yahyaoui *et al.,* 2002). Bahri *et al.* (2009) hypothesized the Middle East as the origin of emerging *Pst* strains to the Western Mediterranean region. Furthermore, Ali *et al.* (2014b) hypothesized the Middle East as source of the aggressive strain PstS2 tolerant to high temperature worldwide spread since 2000 (Milus *et al.*, 2006). The identification of *Berberis* as an alternate host for *P. striiformis* f. sp. *tritici* by Jin *et al.* (2010) and its presence in the Middle East gave more insights to assess its role on the pathogen population diversity and genetic recombination. Moreover, the study of resistance gene diversity of landraces, elite lines and commercial varieties grown in the region helped to understand the selection pressure exerted by the host. One other reason in designing this study was to assess the diversity of two components of *Pst* aggressiveness in the highlight of climate change (Chakraborty, 2013) and the emergence and invasion of aggressive warm-adapted strains worldwide (Hovmøller *et al.*, 2011). Historically, *Pst* occurred mainly in temperate areas (Hau & de Vallavieille-Pope, 2006) and it was anticipated that the pathogen would not survive the harsh Australian conditions (Brown, 1984; Wellings, 2011), American central regions and East states of the Rocky Mountains in the United States (Milus *et al*., 2009). The question here was how the Middle East *Pst* isolates from different regions (warm and cold) in the Middle East would perform under extreme temperatures for spore penetration and incubation period, given that aggressive strains adapted to high temperature were responsible of severe epidemics in different parts of the world (Hovmøller *et al.,* 2011).

The first chapter was designed to postulate the race-specific resistance genes in spring bread wheat ICARDA elite lines, Lebanese varieties and landraces. The infection type observed after inoculation by a set of pathotypes harbouring complementary virulence profiles gave quick detailed information about wheat stripe rust specific resistance genes detectable at the seedling stage. An array of eleven French pathotypes was selected from INRA collection to postulate the presence of ten *Yr*-genes in wheat lines. *Yr1*, *Yr6, Yr7, Yr17* and *Yr27* were the most frequently postulated resistance genes in ICARDA elite lines. Combinations of two *Yr*-genes were more common in ICARDA elite lines than Lebanese varieties and landraces. The lines resistant to all pathotypes figured among ICARDA elite lines and three Lebanese durum wheat varieties. The selectionmwork carried by ICARDA breeders lead to accumulate more resistance gene combinations in ICARDA elite lines than Lebanese varieties. Some lines showed adult plant resistance in both locations, Lebanon and Syria. The ICARDA elite lines combining two *Yr*-genes were more resistant at the adult-plant stage than those having a single *Yr* gene. When combining the genes *Yr6*, *Yr7*, and *Yr9* with others like *Yr1, Yr3* and *Yr17,* the resistance reaction in the field was higher than for one *Yr* gene alone. This finding supported the strategy of combining several genes to protect the plant and reduce the emergence of new specific races. It is well known that more than two *Yr*-resistance genes are required to protect for a long term (Singh *et al*., 2004).

Among tested lines, the highest number of susceptible plants was found among the landraces. However, we observed variation in the proportion of resistant plants between the landraces. Interestingly, one and three landraces showed up to 65% and 45% of resistant plants to the tested pathotypes, respectively. Some plants of a landrace were resistant to “Warrior” race harbouring the largest number of virulences among the tested pathotypes, spread in Europe since 2011. Our findings corroborated with the Chinese study revealing stripe and leaf rust resistance in nine landraces (Zhang, 1995).

Since the deployment of resistance genes is still the most economical and environmentally friendly way to control the disease, it would be very interesting to explore these combinations of specific *Yr* genes expressed since the seedling stage and identify QTLs of adult plant resistance that provide efficient durable resistance (Dedryver *et al*., 2099; Paillard *et al*.,

2012). The combination of both seedling resistance genes protecting the early stage of the crop and adult plant resistance appeared to be the most appropriate strategy.

The second chapter studied the *Pst* diversity in Lebanon and Syria for both virulence and molecular markers for a population sampled in 2010-2011 under high epidemic pressure. *Pst* pathotypes were determined on a robust set of 43 differential hosts including near isogenic lines to get the most precise description of the present virulences/avirulences. We also included the European and world differentials (Johnson *et al*., 1972), having two or three resistance genes for most of them, to confirm the presence of virulences in another genetic background and to be able to compare with other studies using this differential set for a long time (Bayles *et al*., 2000 ; de Vallavieille-Pope *et al*., 2012). We chose four susceptible controls, Morocco, Jupatecco S, Federation and Avocet S. Interestingly, the universal susceptible Avocet S showed avirulence to two Syrian isolates suggesting that Avocet S carried at least one effective gene for resistance to the two isolates. Similarly, the susceptible control cv-Victo showed avirulence to 127 Pakistani isolates from the crop season 2010 (Ali *et al.,* 2014a). This arose the problem of the notion of universal susceptible differential line, one being susceptible in its original area might unexpectedly exhibit resistance in a new region, far from the selection pressure.

New pathotypes are constantly selected as new cultivars are released (Chen *et al*., 2013; de Vallavieille-Pope *et al*., 2012). The 2010-2011 Middle Eastern Pst population was diverse for the pathotypes with ten pathotypes identified among 54 Lebanese and Syrian isolates. All the pathotypes detected in this study have the same common virulences *Vr2, Vr6*, *Vr7*, *Vr9*, and most of them have in addition to *Vr25*, *Vr27* or *VrA.* The virulences *Vr1*, *Vr4*, *Vr5*, *Vr10*, *Vr15*, *Vr32* and *VrSD* were absent in the region, and *Vr*3 detected only once. These virulence profiles were similar to those of PstS1/S2*: Vr2*, *6*, *7*, *8*, *9*, described by Milus *et al.* (2006) and Hovmøller *et al.* (2008). However *Vr8,* described in the PstS2 aggressive strain and in Mediterranean isolates 6E16 (PstS3) (Nazari *et al*., 2008; Mboup *et al*., 2012) was not fixed in this Middle East population. The virulence profiles observed, characterized by the absence of *Vr3* , *VrSD* and *Vr4* was common in this part of world (Bahri *et al*., 2009)although these virulences were fixed in the North-West European populations described at the same period (de Vallavieille-Pope *et al.,* 2012). This study concluded that the region was characterized by a richness of pathotypes as shown in previous studies (Yahayoui *et al*., 2002; Bahri *et al*., 2009). The aggressive PstS2 strain already present in the Middle East in 2004-2005 and prevalent in the 2004-2005 Mediterranean

region (Bahri *et al.,* 2009) was dominant in the studied population. The dominant PstS2 strain found in Lebanon and Syria comprised several variants, having all the fixed virulences *Vr2, 6, 7, 9*, oftenly combined with *Vr17, Vr*25, *Vr27*, *VrA* or *VrSP* .

This high pathotype diversity can be analysed considering the different wheat varieties and landraces which were grown in the area. The prevalent virulences in the CWANA region were coherent with the postulated *Yr*-genes. The resistance genes *Yr6* +, *Yr7* +, and *Yr27* were the most frequently postulated genes on a subset of ICARDA materials, and the Lebanese varieties. The virulences *Vr6* and *Vr7* were fixed in all pathotypes tested and *Vr27* was highly frequent (98%). Virulences to *Yr8* and *Yr17* occurred at intermediate frequencies (55% and 26%, respectively). *Yr8* was absent and *Yr17* postulated only in four elite lines, was rare in the ICARDA and Lebanese germplasm, although *Yr17* was frequent in the North Western European germplasm (Bayles *et al.,* 2000). The widely deployed *Yr3* and *Yr4* in the North Western European varieties (de Vallavieille-Pope *et al.,* 2012) were postulated within the Middle Eastern germplasm although at low frequency, and were not overcome there. Indeed the detected virulences corresponded to resistance genes deployed in the region, suggesting strong host selection pressure (McDonald and Linde, 2002). Therefore, adopting integrated disease management by diversifying cultivars with different resistance genes could be a major strategy in preventing or at least slowing down the emergence and global spread of specific pathotypes by reducing the genetic uniformity of the host (de Vallavieille-Pope *et al.,* 2012).

The population was clonal as shown with 20 microsatellite markers. However the number of 50 MLGs detected were higher than in earlier described clonal populations, as the 2004- 2005 Mediterranean population (Bahri *et al*., 2009) and 1985-2000 North-West European population (Enjalbert *et al*., 2005). The 50 MLGs detected in the population were partitioned in two subpopulations closely related to the Mediterranean Middle East genetic group described by Ali *et al*. (2014b). The MLG-34 closely related to MLG-99 frequent in the PstS2 population described worldwide (Ali *et al*., 2014b) was dominant in the region. The dominant MLG-34 corresponding to PstS2 was present in 60% of the Syrian samples and 22% of Lebanese ones. The studied population was diversified for virulences and multilocus genotypes. Although virulences to the resistance genes *Yr2, 6, 7, 8, 9* were characteristic of PstS2 (Milus *et al*., 2006;

Hovmøller *et al.*, 2011), variants were present with additional virulences to *Yr27* (96%), *Yr25* (98%), *YrA* (93%), *Yr8* (53%), *Yr17* (26%), *YrSP* (15%), or *Yr3* (2%).

These findings suggested that the strains were still evolving and emergence of new variants of the aggressive strains was still on-going with the five evolutionary forces, mutation, genetic drift, gene flow, reproduction/mating system, and selection in the highlight of climate change.

Although the alternate host *Berberis libanotica* and *Berberis cretica* were present in the area (Tohmé and Tohmé, 2002), we did not detect any genetic recombination in the Lebanese and Syrian *Pst* population. Jin *et al*. (2010) and Rodriguez-Algaba *et al.* (2014) showed that *Pst* completed its sexual cycle on different species of *Berberis* sp. in laboratory controlled conditions but the role of the sexual cycle has not been shown under natural field conditions. In the Himalayan region of Pakistan, high genotypic diversity and *Pst* recombinant population structure across locations where *Berberis* sp. was present, were in favour of the existence of sexual reproduction in this region (Ali *et al.*, 2014c). However this recombinant genetic structure was not detected in Lebanon in Syria although *Berberis* was present. This corroborated the situation observed in the Pacific Northwest of the United States where Wang and Chen (2015) showed that there is no synchrony in the prevalence of susceptible leaves of *Berberis* and phenology of *P. striiformis*, i.e. the period when basidiospores are released. Furthermore, Kang *et al.* (2015) found very few *Pst* aecia on *Berberis* in China, although recombinant population was observed in Gansu area (Mboup *et al*., 2009). It would be interesting to lauch an extensive survey in the *Berberis* sp. zone plantations to study in details their role in the sexual phase in the Middle East and to find the appropriate timing for sampling.

Based on the intergovernmental panel on climate change report (2007), it is now generally accepted that average temperatures are rising globally, with rates of change increasing towards the poles, and increasing average temperatures associated with more frequent extreme temperature events. To cope with the environmental conditions changes, living organisms, in order to persist, will need to migrate, tolerate or adapt to locally changed conditions (Chakraborty, 2013). Furthermore, pests and diseases may adapt to changing conditions, for example increasing the breadth of their temperature tolerance or shifting their temperature

optima. This last decade, epidemics have spread in areas considered so-far to be too warm for the pathogen, as south of the United States and Western Australia (Milus *et al*., 2009; Wellings, 2011). It was advocated that the PstS1/S2 strains adapted to warm temperature, first detected by Milus *et al.* (2006) in 2000 in the south of the United States, had migrated worldwide, and reached Australia in 2002 (Wellings, 2011), and then Europe and Asia (Hovmøller *et al*., 2011). The invasion by the clonal lineage PstS1/S2 was mainly responsible of the new epidemics under warmer climate. However, Loladze *et al.* (2014) did not show conclusive evidence for temperature adaptation within old and new invasive *Pst* Australian isolates. Another pathotype 6E16 (PstS3) originating from the Mediterranean area, found in south of France, earlier in the 1980s, was also able to develop at higher temperature than the Northern French pathotypes (Mboup *et al*., 2012).

The question arose whether the aptitude of *Pst* to adapt to warm temperature was selected in rare genotypes and spread clonally through migration, or if this adaptation could be local, and found in different locations. To answer this question, the third chapter was designed and we have selected 26 *Pst* isolates from the Middle East, 13 from warm locations and 13 from cold locations. We have tested two major components of the infection cycle, the infection efficiency estimating the success of infection of the spores, and the latent period, estimating the duration of a generation, under two extreme cold and warm temperatures. We found variation within the Middle Eastern isolates for their response to temperature for both aggressiveness components, but we found no evidence of local adaptation to climatic conditions in infection efficiency and latent period which did not vary significantly between isolates originating from warm versus cold climates. However, isolates originating from cold climatic conditions, having been under a warm dew temperature for spore penetration, had longer latent period under the warm incubation regime than isolates originating from warm climatic conditions. Overall, warm dew temperatures retarded sporulation, but this effect was less marked for isolates from warm climates when incubated under warm conditions; the isolates from warm climates were not as much affected by warm dew temperatures for their infection cycle. The genetic group to which the isolates belonged, i.e. the aggressive PstS1/S2 versus other genetic groups, did not explain significant variation in infection efficiency. However for latent period, we found a significant interaction between genetic group and incubation temperature. The isolates identified as PstS2 sporulated more rapidly than did the other two groups at warm incubation temperatures, which supports the

idea that this genetic group is warm-adapted. The variation was independent of the categorization into new and old group; one Lebanese isolate from Stubbs' collection sampled forty years ago, showed adaptation to high temperature and did not belong to the PstS2 genotypes.

We classified the 26 isolates into five groups. Group 5 with five isolates which were all from warm locations and PstS2 genotypes, had low infection efficiency at warm temperature 20°C and short latent period, and was opposed to Group 1, which had high infection efficiency at 20°C and long latent period. The other three groups were scattered between the groups 1 and 5. Group 4 was the closest to warm-adapted reference isolate included the isolates collected from below 1000 m. Group 2 was the closest to the cold adapted reference isolate having high infection efficiency at 5^0C and comprising isolates from different origins. The adaptation to high temperature of the PstS2 and the other strains was thought to contribute to their prevalence in the warm climate of Middle East, however isolates less well adapted to high temperature were found in the region, therefore they were able to compete there.

The study of the Middle East *Pst* populations, which showed the dominance of PstS2 strains in the region, emphasized the importance of invasive strains. Emergence and spread of new races were already reported the last decades worldwide (Ali *et al.*, 2014b). The capacity of *Pst* uredospores to migrate for long distance gave them advantages in spreading invasive strains and resulting in high yield losses (Brown and Hovmøller, 2002; Hodson, 2011). Already in the early 20th century, the introduction of *Pst* in North America was through migration from North West Europe (Carleton, 1915). The first *Pst* detection in Australia was reported in 1979 from Europe via human travel (Wellings *et al*., 1987). In addition, *Pst* was reported in 1996 in South Africa from an invasive race closely related to Mediterranean-Central Asian population (Boshoff *et al.*, 2002). The detection of closely related pathotypes in 2000 in North America and in Western Australia in 2002 urged to put endeavors together to assess the international *Pst* spread. The strain PstS1 was common in America and PstS2 was common in Eritrea (Red Sea area), West and Central Asia (Hovmøller *et al*., 2008), and Middle East and Mediterranean region (Bahri *et al*., 2009). Similarly, Enjalbert *et al.* (2005) reported in France less aggressive isolates (6E16, PstS3) than PstS1/PstS2 originating from the Mediterranean area (Ali *et al.,* 2014b). More recently, in 2006, a new Triticale-aggressive race emerged in Scandinavia causing

epidemic and yield losses reached up to 100% mainly in organic fields (Hovmøller *et al*., 2015). Since 2011, new invasive races occurred in Europe, the Kranich and Warrior races, which have replaced the existing North West European populations since 2011 (Hubbard *et al*., 2015; Hovmøller *et al*., 2015). The race Warrior reached Morocco and Tunisia in 2013-2014 season causing epidemics in wheat fields (www.wheatrust.org). New invasions and rapid shift were observed in *Pst* population at the regional, national, and international level. The breeders have more challenges ahead to develop new varieties possessing broad range of resistance for the new *Pst* races and their variants.

Conclusion

The present study would provide us with new information generated from the three chapters. The information generated from gene postulation would be very interesting for the breeders in their breeding programs. The second chapter provided us with the prevalent pathotypes in Lebanon and Syria and how the population was evolving in term of virulence and characterizing it with a high number of pathotypes. Based on this, this would give a good idea about the gene deployment by breeders and farmers avoiding planting varieties carrying the same resistance gene over large areas and escaping the boom and bust cycles. The information from our study will help the breeders to understand *Pst* population biology, and eventually to use a better resistance genes deployment for a better management for yellow rust.

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